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4) Ikezaki, 1990, Cancer Letters 49: 115-120.
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Effect of Benzodiazepines and Neurosteroids on Ammonia-Induced Swelling in Cultured Astrocytes

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Astroglial swelling occurs in acute hyperammonemic states, including acute hepatic encephalopathy. In these conditions, the peripheral-type benzodiazepine receptor (PBR), a receptor associated with neurosteroidogenesis, is up-regulated. This study examined the potential involvement of PBRs and neurosteroids in ammonia-induced astrocyte swelling in culture. At low micromolar concentrations, the PBR antagonist PK 11195, atrial natriuretic peptide, and protoporhyrin IX, which are known to interact with the PBR, attenuated (16–100%) the effects of ammonia, whereas the PBR agonists Ro5–4864, diazepam binding inhibitor (DBI_{51-70}), and octadecaneuropeptide exacerbated (10–15%) the effects of ammonia. At micromolar concentrations, diazepam, which interacts with both the PBR and the central-type benzodiazepine receptor (CBR), increased swelling by 11%, whereas flumazenil, a CBR antagonist, had no effect. However, at 100 nM diazepam and flumazenil abrogated ammonia-induced swelling. The neurosteroids dehydroepiandrosterone sulfate, tetrahydroprogesterone, pregnenolone sulfate, and tetrahydrodeoxycorticosterone (THDOC), products of PBR stimulation, at micromolar concentrations significantly enhanced (70%) ammonia-induced swelling. However, at nanomolar concentrations, these neurosteroids, with exception of THDOC, blocked ammonia-induced swelling. We conclude that neurosteroids and agents that interact with the PBR influence ammonia-induced swelling. These agents may represent novel therapies for acute hyperammonemic syndromes and other conditions associated with brain edema and astrocyte swelling. J. Neurosci. Res. 54:673–680, 1998. Published 1998 Wiley-Liss, Inc.†

Key words: ammonia; atrial natriuretic peptide; astrocyte swelling; benzodiazepines; hepatic encephalopathy; neurosteroids; peripheral-type benzodiazepine receptor; protoporphyrin IX

INTRODUCTION

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome that occurs as a complication of severe liver failure. Ammonia is considered to be a major toxic agent in HE (Norenberg, 1977; 1996) in which levels in the central nervous system (CNS) can reach 1–5 mM (Szerb and Butterworth, 1992). Also, endogenous benzodiazepine-like neuropeptides (endozepines) such as diazepam binding inhibitor (DBI) and its metabolite octadecaneuropeptide (ODN) are elevated in HE (Butterworth et al., 1991). Increased levels of these two peptides correlate with the clinical stages of HE (Rothstein et al., 1989; Butterworth et al., 1991). These peptides also interact with peripheral-type benzodiazepine receptors (PBRs) (Costa and Guidotti, 1991; Papadopoulos and Brown, 1995) and stimulate the production of neurosteroids (Papadopoulos and Brown, 1995). Neurosteroids are known to affect γ-aminobutyric acid $(GABA)_A$ and N-methyl-D-aspartate (NMDA) receptors in the CNS (Majewska, 1992; Paul and Purdy, 1992) and may contribute to the pathogenesis of HE (Norenberg, 1996; Norenberg et al., 1997).

HE is also associated with up-regulation of PBRs (Lavoie et al., 1990; Giguere et al., 1992; Itzhak and Norenberg, 1994). Additionally, ammonia has been shown to up-regulate PBRs in cultured astrocytes treated with ammonia (Ducis et al., 1989; Itzhak and Norenberg, 1994). In addition to PBRs (Bender and Hertz, 1985; Itzhak et al., 1993), astrocytes seem to express central-type benzodiazepine receptors (CBRs) (Bormann and Kettenmann, 1988).

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A major cause of death in patients with fulminant hepatic failure is brain edema. Brain swelling seems to be chiefly cytotoxic and occurs principally in astrocytes (Norenberg, 1977; Swain et al., 1991; Cordoba and Blei, 1996). The pathogenesis of such swelling is not known. It is likely that ammonia plays a role because ammonia has been shown to cause swelling in primary cultured astrocytes (Norenberg at al., 1991), in brain slices (Ganz et al., 1989), and in vivo (Voorhies et al., 1983; Blei et al., 1994; Willard-Mack et al., 1996).

To elucidate the role of benzodiazepine receptor ligands in ammonia-induced astrocyte swelling, we examined the effects of various exogenous and endogenous agents that interact with astrocytic benzodiazepine receptors. We also investigated the action of neurosteroids that are products of PBR stimulation. Some of this study has been presented in preliminary form (Norenberg and Bender, 1994; Norenberg et al., 1997).

MATERIALS AND METHODS

Materials

DBI (DBI_{51-70}), ODN (DBI_{33-50}), dehydroepisoandrosterone 3 sulfate (5-androsten-3β-ol-17-one sulfate) sodium salt (DHEAS), 5α-pregnane-3α,21-diol-20-one (allotetrahydrodeoxycorticosterone) (THDOC), 5α-pregnan-3β-ol-20-one (THP), clonazepam, diazepam, and protoporphyrin IX (PpIX) were purchased from Sigma Chemical (St. Louis, MO). 5-Pregnen-3-β-ol-20 sulfate sodium salt (PS) and PK11195 were obtained from Research Biochemicals, Inc. (Natick, MA). Ro5–4864 was purchased from Biomol Research Labs Inc. (Plymouth Meeting, PA) and atriopeptin III (ANP) from Peninsula Laboratories, Inc. (Belmont, CA). Ro15–1788 (flumazenil) was a gift from Dr. Peter Sorter of Hoffmann-LaRoche, (Nutley, NJ). Methyl-D-glucose, 3-O[Methyl-^{3}H)] (86.7 Ci/mmol) was purchased from New England Nuclear Research Products (Boston, MA). All other reagents were of analytical grade.

Cell Culture

Astrocytic cell cultures were prepared from neonatal (1–2-day-old) rat cerebral cortices and maintained in primary culture as previously described by Ducis et al. (1990). Briefly, the tissue was dissociated and 0.5×10^6 cells were plated in 35-mm dishes. The cells were grown for at least 3–4 weeks at 37°C in a humidified atmosphere containing 5% CO_2/95% air before being used for biochemical studies. After 2 weeks, the cells were maintained with 0.5 mM dibutyryl cyclic adenosine monophosphate (AMP). Based on the immunohistochemistry of glial fibrillary acidic protein and glutamine synthetase, at least 95–98% of the cell population was astrocytes.

Intracellular Water Space Measurements

Astrocytic cell volume and its regulation were studied by measuring the intracellular water space [3-O-(methyl-^{3}H)-D-glucose (3-OMG) space] using the method of Kletzien et al. (1975). This commonly used method for measuring cell volume gives excellent, reproducible results and is particularly useful in assessing relative changes in cell volume. Briefly, following the removal of Dulbecco's Modified Eagle's Medium (DMEM) growth media, the cells were incubated in bicarbonate-buffer DMEM media, which contained 1 mM 3-OMG and [^{3}H]-3-OMG (0.6 μCi/ml) in the presence or absence of various agents. The media contained glucose for energy supply. The presence or absence of glucose had no effect on the equilibration of 3-OMG for up to 3 hr (Jay et al., 1990). Treatment of astrocytes with benzodiazepine receptor ligands and neurosteroids had no effect on 3-OMG transport. Agents were added for incubation time periods ranging from 6 to 72 hr. Such treatment did not affect cell viability, as determined with the lactate dehydrogenase-release method (Koh and Choi, 1987). After incubation, the cells were quickly washed six times with ice-cold 0.29 M sucrose solution containing Tris nitrate (pH 7.4), 0.5 mM $Ca(NO_3)_2$, and 0.1 mM phloretin. Cells were then solubilized in 1 N NaOH and aliquots were used for scintillation counting and protein content (Lowry et al., 1951). The amount of radioactivity was transformed to the amount representing the intracellular water space, and the results were expressed as microliter of water per milligram of protein.

RESULTS

Effect of Agents Interacting with Benzodiazepine Receptors on Astrocyte Cellular Volume

Astrocyte cell volume increased by 49–65% after 24–72 hr of treatment with 5 mM NH_4Cl. Ro5–4864 (agonist of the PBR) (10 μM) significantly ($P < 0.02$) increased ammonia-induced astrocyte swelling by 10–16% after 36–72 hr of treatment (Fig. 1A). PK 11195 (antagonist of the PBR) (10 μM) significantly attenuated ($P < 0.03$) such swelling by 12–17% (Fig. 1A). The effects of Ro5–4864 and PK 11195 on their own had no statistically significant effect on cell volume (Fig. 1A). Diazepam (mixed-type benzodiazepine receptor agonist) (10 μM) and flumazenil (antagonist of the CBR) (10 μM) alone did not significantly affect astrocyte cell volume. However, diazepam significantly enhanced ammonia-induced astrocyte swelling by 11–17% on days 2 and 3, whereas flumazenil had no significant effect (Fig. 1B).

Figure 2 shows the effect of Ro5–4864, PK 11195, diazepam, and flumazenil (100 nM) on ammonia-induced swelling. Swelling after 1-day treatment was blocked by diazepam and flumazenil, whereas Ro5–4864 and PK 11195 did not exert any significant effect. After 2- and

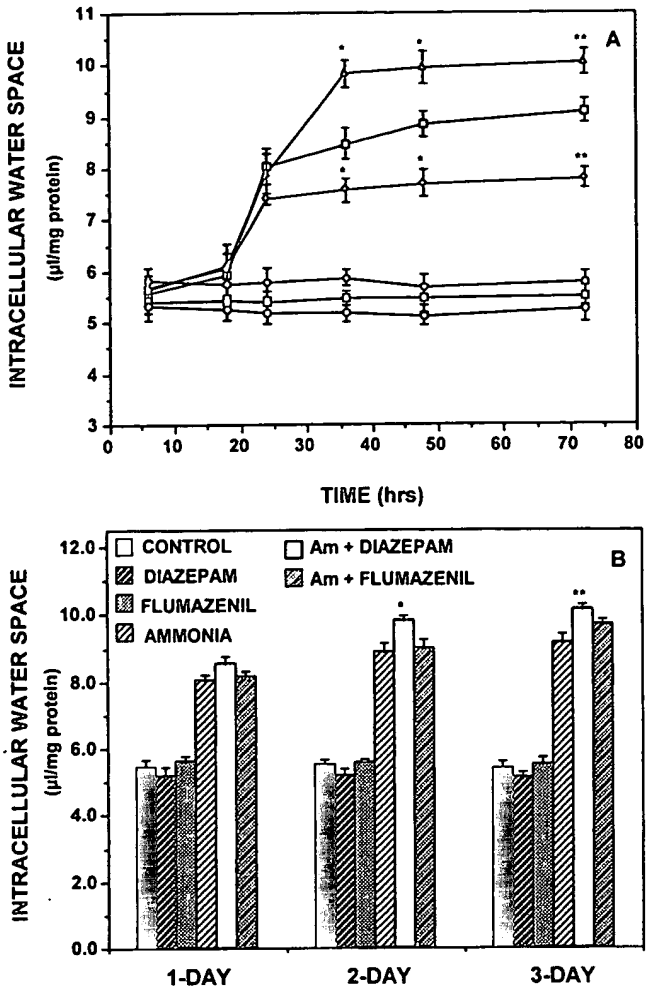

Fig. 1. A: Effect of micromolar concentrations of Ro5–4864 and PK 11195 on cell volume and ammonia-induced astrocyte swelling. Astrocytes were exposed to DMEM in the absence (control; filled squares) or presence of 10 µM Ro5–4864 (filled circles), 10 µM PK 11195 (open circles), 5 mM NH_4Cl (open squares), 5 mM ammonia with 10 µM Ro5–4864 (filled triangles), and 5 mM ammonia with 10 µM PK 11195 (filled diamonds) for various time periods, and cell volume was determined. B: Effect of micromolar concentrations of diazepam and flumazenil on astrocyte cell volume and ammonia (Am)-induced swelling. Concentrations of diazepam and flumazenil were 10 µM, and NH_4Cl was 5 mM. Results are expressed as mean ± S.E.M. of six separate determinations. $*P < 0.05$; $**P < 0.01$ significantly different from ammonia-induced swelling, as assessed by analysis of variance and Bonferroni post hoc comparisons.

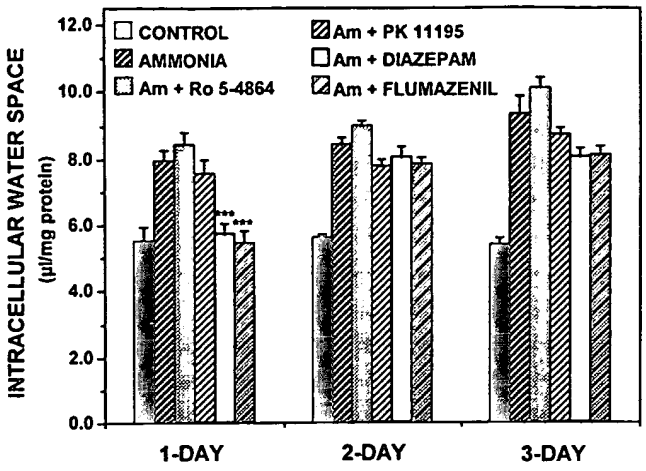

Fig. 2. Effect of various agents that interact with benzodiazepine receptors at nanomolar concentrations on ammonia-induced astrocyte swelling. Astrocytes were exposed to DMEM containing 5 mM ammonia (Am) with or without 100 nM Ro5–4864, PK 11195, diazepam, and flumazenil for 1–3 days, and cell volume was determined. Results are expressed as mean ± S.E.M. of six separate determinations. $***P < 0.001$ significantly different from ammonia-induced swelling, as determined by analysis of variance and Bonferroni post hoc comparisons.

3-day treatment, none of these agents had any significant effect on ammonia-induced swelling. These compounds on their own at 100 nM also did not significantly affect astrocyte cell volume after 1–3-day treatment.

DBI_{51-70} at a concentration normally found in brain (15 µM) (Costa and Guidotti, 1991) and ODN DBI_{33-50}, agents that interact with PBRs and CBRs (Costa and Guidotti, 1991; Papadopoulos and Brown, 1995) significantly increased astrocyte cell volume by 30% and enhanced ammonia-induced astrocyte swelling by 10–15% (Fig. 3).

ANP (1 µM), a peptide that also interacts with astrocytic benzodiazepine receptors (Bender and Hertz, 1987), significantly decreased ammonia-induced astrocyte swelling by 50% (Fig. 4). ANP alone caused cell shrinkage (16–25%).

Porphyrins are known to interact with the PBR at nanomolar concentrations. Of the various porphyrins examined, PpIX had the highest affinity (Snyder et al., 1987). PpIX at 100 nM inhibited ammonia-induced swelling by 11%, which was not statistically significant. However, at 1 µM concentration, such inhibition was statistically significant (20–24%). PpIX alone had no significant effect on astrocyte cell volume after 1–3-day treatment (Fig. 5).

Effect of Neurosteroids on Astrocyte Cell Volume

Agonists of the PBR have been shown to stimulate neurosteroid synthesis (Kruger and Papadopoulos, 1992; Papadopoulos and Brown, 1995). Neurosteroids, such as PS, THP, THDOC, and DHEAS were thus tested for their effect on cell volume and ammonia-induced astrocyte swelling.

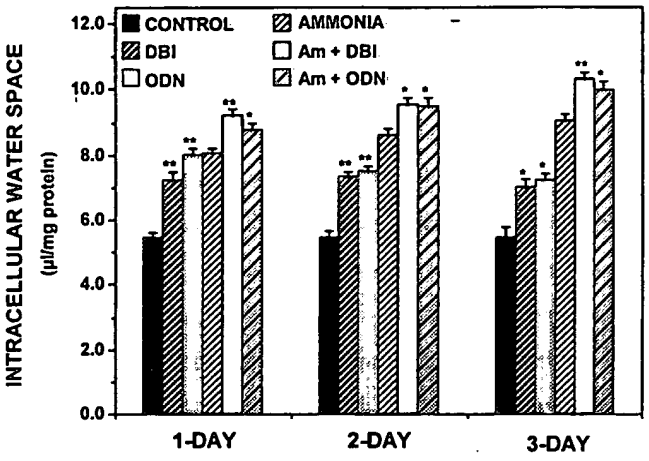

Fig. 3. Effect of DBI and ODN on astrocyte cell volume and ammonia (Am)-induced swelling. Astrocytes were exposed to DMEM in the absence (control) or presence of 15 µM DBI, 15 µM ODN, 5 mM ammonia, 5 mM ammonia with 15 µM DBI, and 5 mM ammonia with 15 µM ODN for 1–3 days, and cell volume was determined. Results are expressed as mean ± S.E.M. of six separate determinations. $*P < 0.05$; $**P < 0.01$ significantly different from control or from ammonia-induced swelling, as determined by analysis of variance and Bonferroni post hoc comparisons.

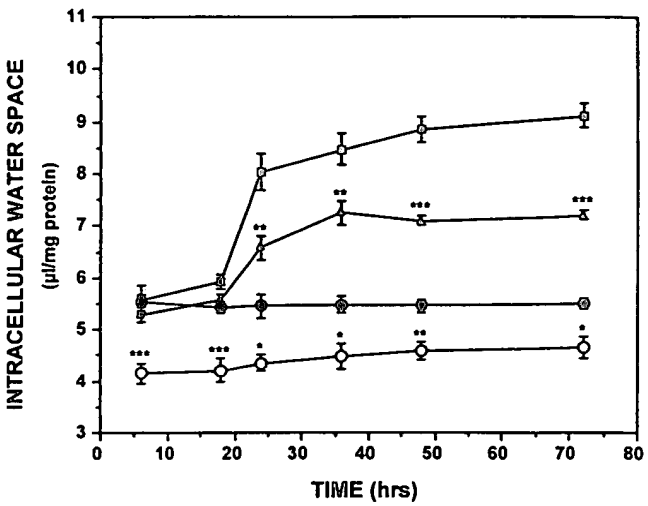

Fig. 4. Effect of ANP on cell volume and ammonia-induced astrocyte swelling. Astrocytes were exposed to DMEM in the absence (control; filled circles) or presence of 5 mM ammonia (filled squares), 5 mM ammonia with 1 µM ANP (filled triangles), and 1 µM ANP (open circles) for various time periods, and cell volume was determined. Results are expressed as mean ± S.E.M. of six separate determinations. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ significantly different from ammonia-induced swelling or from control, as determined by analysis of variance and Bonferroni post hoc comparisons.

Figure 6 shows the effects of neurosteroids at 100 nM concentration on ammonia-induced swelling. After 1-day treatment, swelling was blocked by DHEAS, THP, and PS but not by THDOC. Neurosteroids had no effect on ammonia-induced swelling after 2-day treatment. After 3-day treatment, there was a slight increase in ammonia-induced swelling, but the effect was not statistically significant. Neurosteroids (100 nM) alone had no significant effect on cell volume after 1–3-day treatment.

The above neurosteroids at 10 µM and THDOC at 1 µM alone increased astrocyte cell volume by 9–20% after 1–3-day treatment (Fig. 7A). Neurosteroids further exacerbated ammonia-induced astrocyte swelling by 19% ($P < 0.001$; n = 9) after 1 day, by 19–53% ($P < 0.001$; n = 9) after 2 days, and by 50–68% ($P < 0.001$; n = 9) after 2 days (Fig. 7B).

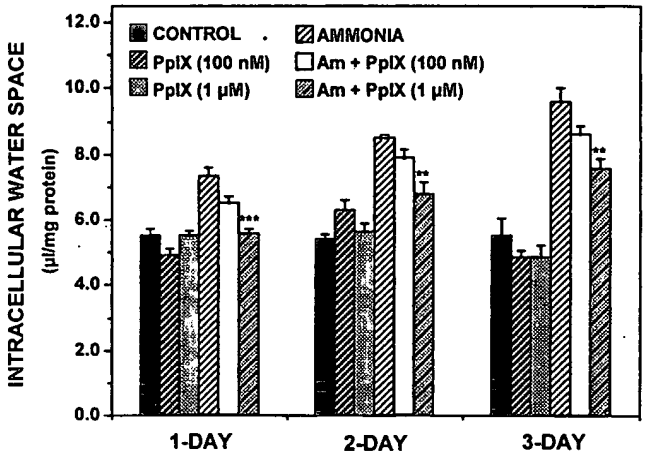

Fig. 5. Effect of PpIX on cell volume and ammonia (Am)-induced astrocyte swelling. Astrocytes were exposed to DMEM in the absence (control) or presence of 100 nM or 1 µM PpIX, or 5 mM ammonia, 5 mM ammonia with 100 nM PpIX, and 5 mM ammonia with 1 µM PpIX for 1–3 days, and cell volume was determined. Results are expressed as mean ± S.E.M. of six separate determinations. $**P < 0.01$; $***P < 0.001$ significantly different from ammonia-induced swelling, as determined by analysis of variance and Bonferroni post hoc comparisons.

DISCUSSION

Brain edema is a major cause of death in patients with fulminant hepatic failure, in which the astrocyte is the main cellular compartment that is subjected to swelling (Norenberg, 1977; Swain et al., 1991; Blei et al., 1994; Cordoba and Blei, 1996). The pathogenesis of such swelling most likely reflects the exposure of astrocytes to toxic substances, particularly ammonia (Cordoba and Blei, 1996). Ammonia has been shown to cause astrocyte

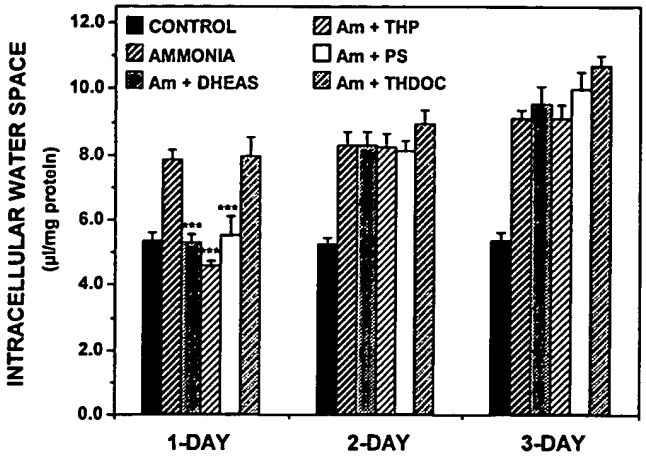

Fig. 6. Effect of neurosteroids at nanomolar concentrations on ammonia (Am)-induced astrocyte swelling. Astrocytes were exposed to DMEM in the absence (control) or presence of 5 mM ammonia, 5 mM ammonia with 100 nM DHEAS, 5 mM ammonia with 100 nM THP, 5 mM ammonia with 100 nM PS, and 5 mM ammonia with 100 nM THDOC for 1–3 days, and cell volume was determined. Results are expressed as mean ± S.E.M. of six separate determinations. ***$P < 0.001$ significantly different from ammonia-induced swelling, as determined by analysis of variance and Bonferroni post hoc comparisons.

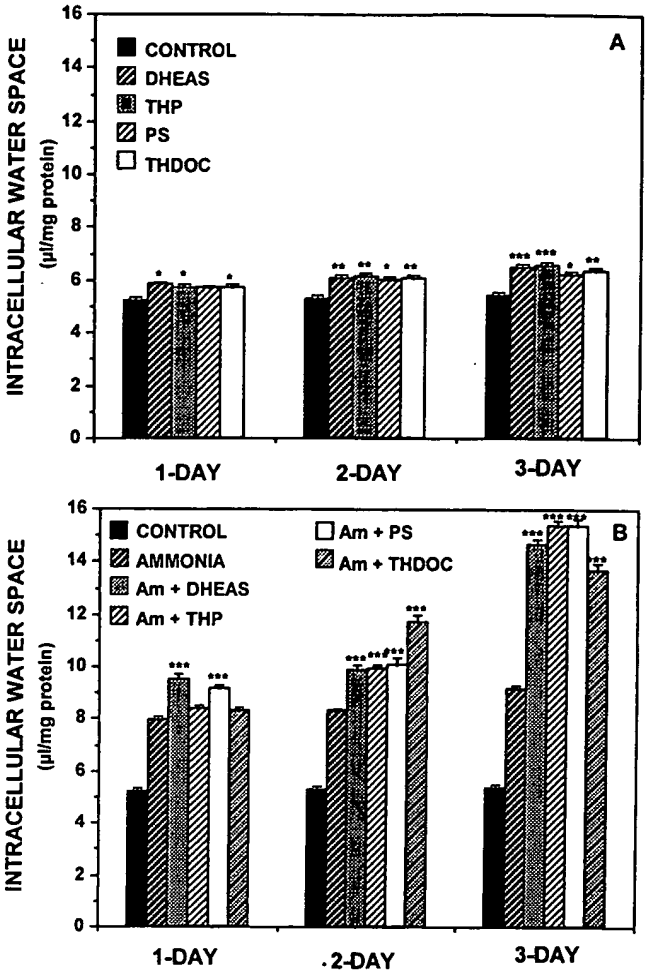

Fig. 7. Effect of neurosteroids at micromolar concentrations on cell volume (A) and ammonia (Am)-induced astrocyte swelling (B). Astrocytes were exposed to DMEM in the absence (control) or presence of 10 µM DHEAS, THP, PS or 1 uM THDOC (A); or 5 mM ammonia, 5 mM ammonia with 10 µM DHEAS, 5 mM ammonia with 10 µM THP, 5 mM ammonia with 10 µM PS, and 5 mM ammonia with 1 µM THDOC (B) for 1–3 days, and cell volume was determined. Results are expressed as mean ± S.E.M. of nine separate determinations. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ significantly different from control (A) or from ammonia-induced swelling (B), as determined by analysis of variance and Bonferroni post hoc comparisons.

swelling in culture (Norenberg et al., 1991) and in brain slices (Ganz et al., 1989). The mechanism by which ammonia causes swelling is unknown. It has been proposed that ammonia-induced swelling may be a result of increased intracellular glutamine levels that increase the osmotic load and lead to cell swelling (Brusilow and Traystman, 1986). Methionine sulfoximine, an inhibitor of glutamine synthetase, is known to prevent ammonia-induced swelling (Takashi et al., 1991; Blei et al., 1994; Norenberg and Bender, 1994; Norenberg et al., 1995; Willard-Mack et al., 1996).

Benzodiazepine Receptor Ligands and Ammonia-Induced Astrocyte Swelling

HE and hyperammonemia are associated with increased levels of endogenous benzodiazepines ligands in cerebrospinal fluid and brain tissue from patients with HE (Mullen et al., 1988). It has also been shown that PBRs are up-regulated in HE (Lavoie et al., 1990) and in ammonia-treated cultured astrocytes (Ducis et al., 1989; Itzhak and Norenberg, 1994). More recently, an increase in neurosteroids, presumably from the up-regulation of PBRs, has been seen in hyperammonemic conditions (Itzhak et al., 1995; Norenberg et al., 1997). Because of the potential involvement of benzodiazepines and neurosteroids in the pathogenesis of HE and hyperammonemia, we explored the possibility that they may also contribute to the astrocyte swelling characteristic of these conditions.

Our results show that agents that interact with the PBR, as well as neurosteroids (products of PBR stimulation), influence ammonia-induced astrocyte swelling. Swelling was enhanced by micromolar levels of PBR agonists such as Ro5–4864, diazepam, as well as by endogenous benzodiazepine-like peptides such as DBI

and ODN. On the other hand, ammonia-induced swelling was diminished by a PBR antagonist, PK 11195, whereas, at micromolar concentrations, flumazenil, a CBR antagonist, had no effect. However, diazepam, which interacts with both PBR and CBR, and flumazenil at concentrations of 100 nM blocked ammonia-induced swelling after 1-day treatment. Astrocytes express CBRs (Bormann and Kettenmann, 1988), and flumazenil has been shown to have a beneficial effect in clinical trials in patients with HE (Ferenci et al., 1989; Pomier-Layrargues et al., 1994).

ANP, which inhibits [^{3}H]-diazepam binding from astrocytes (Bender and Hertz, 1987), attenuated ammonia-induced swelling, thus behaving like the PBR antagonist PK 11195. ANP has been shown to reduce glial cell volume (Latzkovits et al., 1993), decrease cell volume of rabbit atrial and ventricular myocytes (Clemo and Baumgarten, 1991), and reduce ischemic brain edema (Naruse et al., 1991). Although the inhibition of ammonia-induced astrocyte swelling by ANP may be caused by the inhibition of the PBR, it also could be a result of direct effects of ANP on various ionic fluxes. ANP has been shown to reduce cell volume by inhibiting the $Na^+/K^+/2Cl^-$ cotransporter in myocytes (Clemo and Baumgarten, 1991), to accelerate efflux of Na^+ from brain (Doczi et al., 1987) and to inhibit brain water and Na^+ accumulation in ischemic brain injury (Naruse et al., 1991).

Another endogenous agent that interacts with the PBR is PpIX (Snyder et al., 1987). In our study PpIX also attenuated ammonia-induced swelling, behaving in manner similar to that of the PBR antagonist PK 11195 and ANP.

ODN, DBI, and ANP independently affected cellular volume. Therefore, these agents may have potential therapeutic applications not only in hyperammonemia but also in other conditions associated with brain edema and astrocyte swelling.

Our results suggest that agents that interact with PBRs have effects on cellular water homeostasis. This view is consistent with the observation that thiazide-like compounds, and other distally acting diuretics, inhibit Ro5–4864 binding with a rank order of potencies similar to their enhancement of natriuresis (Lukeman and Fanestil, 1987). Also, diuretics, like furosemide, acetazolamide, and hydrochlorothiazide produce significant increases in the density of PBRs in the renal cortex (Lukeman et al., 1988). Up-regulation in PBR densities may depend to some extent on the degree to which each of the these diuretics interacts directly with the PBRs. Alternatively, PBR up-regulation could result from secondary adaptive mechanisms associated with altered metabolic or electrolyte status. It is noteworthy that hypoosmotic stress up-regulates PBRs in astrocytes (Itzhak et al., 1994). The time course of ammonia-induced astrocyte swelling shown in this study correlates with the time course of ammonia-induced up-regulation of PBRs (Itzhak and Norenberg, 1994).

Neurosteroids and Ammonia-Induced Astrocyte Swelling

The PBR is involved in the production of neurosteroids by promoting the delivery of cholesterol across the outer mitochondrial membrane (Krueger and Papadopoulos, 1992; Papadopoulos and Brown, 1995). Astroglia have been shown to synthesize neurosteroids (Kabbadj et al., 1993). The up-regulation of the PBR associated with HE was recently shown to be associated with an increase in brain levels of neurosteroids (Itzhak et al., 1995; Norenberg et al., 1997).

Our results show that neurosteroids (DHEAS, THP, and PS) at nanomolar concentrations attenuated ammonia-induced swelling, after 1-day treatment. However, these neurosteroids did not affect astrocyte swelling after 2 or 3 days of ammonia treatment. On the other hand, at micromolar concentrations, they enhanced ammonia-induced astrocyte swelling. The most potent effect was observed with THDOC (1 μM), whereas other neurosteroids significantly affected cell volume and ammonia-induced swelling at 10 μM.

These low micromolar concentrations of neurosteroids are above the physiological concentrations found in the brain, which are usually in the low nanomolar range (Backstrom et al., 1990). In acute liver failure and hyperammonemia, we observed increases in pregnenolone, THDOC, and THP. Pregnenolone concentrations detected in whole brain homogenates reached 42–60 nM, THP reached 281 nM, and THDOC reached 65 nM (Itzhak et al., 1995; Norenberg et al., 1997). However, the precise concentration of these neurosteroids at critical sites in the CNS are not known. Nonetheless, our studies suggest that neurosteroids, which are elevated in hyperammonemic states, may have a protective effect on ammonia-induced swelling (nanomolar concentrations) or contribute to astrocyte swelling (micromolar concentrations).

Benzodiazepines and neurosteroids had bimodal effects on ammonia-induced astrocyte swelling. Neurosteroids at nanomolar concentrations diminished swelling, whereas at micromolar concentrations they exacerbated swelling. This dual pattern of neurosteroid action has also been observed on the $GABA_A$ and NMDA receptors, where neurosteroids act as positive or negative modulators, depending on their concentrations(Majewska, 1992; Paul and Purdy, 1992). The mechanism for this concentration-dependent effect is not known. The bimodal effects of benzodiazepines on ammonia-induced effects can perhaps be explained by their stimulation of neurosteroid synthesis.

The inhibitory effects of nanomolar levels of neurosteroids on ammonia-induced swelling were only ob-

served on day 1 after treatment, whereas, on days 2 and 3, neurosteroids exacerbated swelling. These effects can possibly be explained by the known nongenomic and genomic actions of neurosteroids (Majewska, 1992). At early times of treatment, neurosteroids exert their effects by direct nongenomic actions (possibly allosteric effects). The later effects are mediated by binding to specific intracellular receptors that are present in astrocytes (Jung-Testas et al., 1992). The dual mechanisms of neurosteroid action seem to have opposing effects. Another plausible explanation for the dual effect of neurosteroids could be due to changes in receptor sensitivity (down- or up-regulation).

We have shown that the PBR antagonist, such as PK 11195, and ANP and PpIX, which interact with the PBR, diminished ammonia-induced swelling, whereas PBR agonists such as Ro5–4864, diazepam, DBI, and ODN at micromolar levels and neurosteroids at micromolar levels enhanced the ammonia-induced swelling. Neurosteroids such as DHEAS, THP, and PS, and diazepam and flumazenil at 100 nM concentrations prevented ammonia-induced swelling after 1-day treatment. In summary, PBR ligands and neurosteroids, depending on their concentration, influence ammonia-induced swelling and may lead to new therapeutic approaches toward the treatment of brain swelling associated with hyperammonemic syndromes and other conditions associated with brain edema and astrocyte swelling.

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SYSTEM:OS  - DIALOG OneSearch
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*File 155: reloaded, note accession numbers changed.
  File 434:SciSearch(R) Cited Ref Sci  1974-1989/Dec
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  File  55:Biosis Preiviews(R)  1993-1999/Jul W4
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*File  55: File is reloaded. Accession number changed.

      Set  Items  Description
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? s peripheral(w)type(5n)benzodiazepine(5n)receptor? ?

          319828  PERIPHERAL
          853185  TYPE
           31270  BENZODIAZEPINE
          889466  RECEPTOR? ?
      S1     574  PERIPHERAL(W)TYPE(5N)BENZODIAZEPINE(5N)RECEPTOR? ?
? s antagonist

      S2  145689  ANTAGONIST
? s s1 and s2

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          145689  S2
      S3     105  S1 AND S2
? s antagonist? ?

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          437722  S4
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 7/3,K,AB/85     (Item 85 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

04421321   84294649
  Electrophysiological  and  pharmacological characterization of peripheral
benzodiazepine receptors in a guinea pig heart preparation.
  Mestre M; Carriot T; Belin C; Uzan A; Renault C; Dubroeucq MC; Gueremy C;
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Le Fur G
Life Sci (ENGLAND) Aug 27 **1984**, 35 (9) p953-62, ISSN 0024-3205
Journal Code: L62
Languages: ENGLISH
Document type: JOURNAL ARTICLE
RO5-4864 decreased in a dose-dependent manner, from 3 X 10(-9) M to 3 X 10(-6) M, the duration of intracellular action potential and the contractility in a guinea pig preparation. Diazepam was less effective and clonazepam inactive. The effects of RO5-4864 were GABA-independent and antagonized by PK 11195 but not by the selective **antagonist** of the brain type **benzodiazepine receptors** RO15-1788. These results show the pharmacological relevance of **peripheral type benzodiazepine** binding sites at the cardiac level.

Aug 27 **1984**,
... RO5-4864 were GABA-independent and antagonized by PK 11195 but not by the selective **antagonist** of the brain type **benzodiazepine receptors** RO15-1788. These results show the pharmacological relevance of **peripheral type benzodiazepine** binding sites at the cardiac level.

7/3,K,AB/86 (Item 86 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04419129 84261951
Diazepam increases membrane fluidity of rat hippocampus synaptosomes.
Mennini T; Ceci A; Caccia S; Garattini S; Masturzo P; Salmona M
FEBS Lett (NETHERLANDS) Jul 23 **1984**, 173 (1) p255-8, ISSN 0014-5793 Journal Code: EUH
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Diazepam in vitro produced a concentration-dependent increase of membrane fluidity in crude synaptic membranes from rat hippocampus, but not cerebellum. Similar effects were obtained with higher concentrations of Ro 15-1788 and PK 11195, while zopiclone was completely inactive. In vivo acute treatment with diazepam and Ro 15-1788 gave results similar to those in vitro. The specific benzodiazepine **antagonist** also significantly increased membrane fluidity and was not able to reverse diazepam's effect. The data are discussed in terms of a possible role of protein kinase inhibition by the drugs not mediated by the 'central' or '**peripheral**' **type** of **benzodiazepine receptors**.

Jul 23 **1984**,
... diazepam and Ro 15-1788 gave results similar to those in vitro. The specific benzodiazepine **antagonist** also significantly increased membrane fluidity and was not able to reverse diazepam's effect. The...

... possible role of protein kinase inhibition by the drugs not mediated by the 'central' or '**peripheral**' **type** of **benzodiazepine receptors**.

7/3,K,AB/87 (Item 87 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04390290 83142291
Purines interact with 'central' but not 'peripheral' benzodiazepine binding sites.
Skerritt JH; Chen Chow S; Johnston GA; Davies LP
Neurosci Lett (NETHERLANDS) Dec 23 **1982**, 34 (1) p63-8, ISSN 0304-3940 Journal Code: N7N
Languages: ENGLISH

Document type: JOURNAL ARTICLE
The effects of several purines and the purine uptake inhibitor, dipyridamole, on the binding, to rat brain membranes, of 4 benzodiazepines with different pharmacological specificities were studied. While all purines tested displaced the binding of [3H](+)-3-methyl-clonazepam and [3H]Ro15-1788, selective agonist and **antagonist** ligands respectively for 'central' **benzodiazepine receptors**, purines had little or no affinity for [3H]Ro5-4864 '**peripheral**'-**type** binding sites in brain, heart or kidney. These results suggest that purines interact with a pharmacologically relevant class of central benzodiazepine 'receptors', and not with central and peripheral 'acceptor' sites labelled by the benzodiazepine Ro5-4864.

Dec 23 **1982**,
... displaced the binding of [3H](+)-3-methyl-clonazepam and [3H]Ro15-1788, selective agonist and **antagonist** ligands respectively for 'central' **benzodiazepine receptors**, purines had little or no affinity for [3H]Ro5-4864 '**peripheral**'-**type** binding sites in brain, heart or kidney. These results suggest that purines interact with a ...

7/3,K,AB/88 (Item 1 from file: 434)
DIALOG(R)File 434:SciSearch(R) Cited Ref Sci

07385725 Genuine Article#: D0281 Number of References: 19
Title: PK-11195, AN **ANTAGONIST** OF **PERIPHERAL TYPE** **BENZODIAZEPINE RECEPTORS**, MODULATES BAY K8644 SENSITIVE BUT NOT BETA-**RECEPTOR** OR H-2-RECEPTOR SENSITIVE VOLTAGE OPERATED CALCIUM CHANNELS IN THE GUINEA-PIG HEART
Author(s): MESTRE M; CARRIOT T; NELIAT G; UZAN A; RENAULT C; DUBROEUCQ MC; GUEREMY C; DOBLE A; LEFUR G
Corporate Source: PHARMUKA LABS,GRP RHONE POULENC SANTE,35 QUAI MOULIN CAGE/F-92231 GENNEVILLIERS//FRANCE/
Journal: LIFE SCIENCES, **1986**, V39, N4, P329-339
Language: ENGLISH Document Type: ARTICLE

Title: PK-11195, AN **ANTAGONIST** OF **PERIPHERAL TYPE** **BENZODIAZEPINE RECEPTORS**, MODULATES BAY K8644 SENSITIVE BUT NOT BETA-**RECEPTOR** OR H-2-RECEPTOR SENSITIVE VOLTAGE OPERATED CALCIUM CHANNELS IN THE GUINEA-PIG HEART
, **1986**

7/3,K,AB/89 (Item 2 from file: 434)
DIALOG(R)File 434:SciSearch(R) Cited Ref Sci

06462297 Genuine Article#: AHF57 Number of References: 31
Title: **PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS** - AUTORADIOGRAPHIC LOCALIZATION IN WHOLE-BODY SECTIONS OF NEONATAL RATS
Author(s): ANHOLT RRH; DESOUZA EB; OSTERGRANITE ML; SNYDER SH
Corporate Source: JOHNS HOPKINS UNIV,SCH MED,DEPT NEUROSCI,725 N WOLFE ST/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT PHARMACOL & EXPTL THERAPEUT/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT PSYCHIAT & BEHAV SCI/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT PEDIAT/BALTIMORE//MD/21205
Journal: JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, **1985**, V233, N2, P517-525
Language: ENGLISH Document Type: ARTICLE

Title: **PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS** - AUTORADIOGRAPHIC LOCALIZATION IN WHOLE-BODY SECTIONS OF NEONATAL RATS
, **1985**

Research Fronts: 85-2902 006 (BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF BENZODIAZEPINE RECEPTOR **ANTAGONISTS** AND AGONISTS)
85-7970 001 (QUANTITATIVE AUTORADIOGRAPHIC LOCALIZATION AND DISTRIBUTION OF MUSCARINIC, OPIATE, SEROTONIN, DOPAMINE...

7/3,K,AB/90 (Item 3 from file: 434)
DIALOG(R)File 434:SciSearch(R) Cited Ref Sci

06420316 Genuine Article#: AFV67 Number of References: 21
Title: DEPLETION OF **PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS** AFTER HYPOPHYSECTOMY IN RAT ADRENAL-GLAND AND TESTIS
Author(s): ANHOLT RRH; DESOUZA EB; KUHAR MJ; SNYDER SH
Corporate Source: JOHNS HOPKINS UNIV,SCH MED,DEPT NEUROSCI,725 N WOLFE ST/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT PHARMACOL & EXPTL THERAPEUT/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT PSYCHIAT & BEHAV SCI/BALTIMORE//MD/21205
Journal: EUROPEAN JOURNAL OF PHARMACOLOGY, **1985**, V110, N1, P41-46
Language: ENGLISH Document Type: ARTICLE

Title: DEPLETION OF **PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS** AFTER HYPOPHYSECTOMY IN RAT ADRENAL-GLAND AND TESTIS
, **1985**
Research Fronts: 85-2902 007 (BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF BENZODIAZEPINE RECEPTOR **ANTAGONISTS** AND AGONISTS)
85-7970 001 (QUANTITATIVE AUTORADIOGRAPHIC LOCALIZATION AND DISTRIBUTION OF MUSCARINIC, OPIATE, SEROTONIN, DOPAMINE...

7/3,K,AB/91 (Item 4 from file: 434)
DIALOG(R)File 434:SciSearch(R) Cited Ref Sci

06222613 Genuine Article#: AAQ17 Number of References: 49
Title: **PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS** IN ENDOCRINE ORGANS - AUTORADIOGRAPHIC LOCALIZATION IN RAT PITUITARY, ADRENAL, AND TESTIS
Author(s): DESOUZA EB; ANHOLT RRH; MURPHY KMM; SNYDER SH; KUHAR MJ
Corporate Source: JOHNS HOPKINS UNIV,SCH MED,DEPT NEUROSCI,725 N WOLFE ST/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT PHARMACOL & EXPTL THERAPEUT/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT PSYCHIAT/BALTIMORE//MD/21205; JOHNS HOPKINS UNIV,SCH MED,DEPT BEHAV SCI/BALTIMORE//MD/21205
Journal: ENDOCRINOLOGY, **1985**, V116, N2, P567-573
Language: ENGLISH Document Type: ARTICLE

Title: **PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS** IN ENDOCRINE ORGANS - AUTORADIOGRAPHIC LOCALIZATION IN RAT PITUITARY, ADRENAL, AND TESTIS
, **1985**
Research Fronts: 85-2902 006 (BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF BENZODIAZEPINE RECEPTOR **ANTAGONISTS** AND AGONISTS)
85-7970 002 (QUANTITATIVE AUTORADIOGRAPHIC LOCALIZATION AND DISTRIBUTION OF MUSCARINIC, OPIATE, SEROTONIN, DOPAMINE...

7/3,K,AB/92 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Preiviews(R)

09137319 BIOSIS NO.: 199497145689
Potentiation of 5-methoxy-N,N-dimethyltryptamine-induced head-twitches by diazepam: Evidence for involvement of adenosine uptake inhibition.

AUTHOR: Moser Paul C
AUTHOR ADDRESS: Marion Merrell Dow Res. Inst., 16 rue d'Ankara, F-67000 Strasbourg, France

JOURNAL: Drug Development Research 30 (4):p213-218 1993
ISSN: 0272-4391
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

8/19/99

ABSTRACT: Previous work shows that benzodiazepines potentiate head-twitches induced by 5-HT agonists and that this action is not mediated via the GABA receptor complex. In the present study the involvement of adenosinergic mechanisms in this effect has been examined, as in addition to their actions at the GABA receptor, benzodiazepines also inhibit adenosine uptake. The adenosine **antagonists** caffeine (0.3-30 mg/kg ip) and 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (0.03-7 mg/kg ip) dose-dependently inhibited the ability of diazepam (4 mg/kg ip) to potentiate head-twitches induced by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT; 2.5 mg/kg ip) without affecting head-twitches induced by S-MeODMT alone at a higher dose (10 mg/kg), which induced a similar number of head-twitches to the combination of 5-MeODMT and diazepam. The adenosine uptake inhibitors papaverine, mioflazine, and dilazep all potentiated head-twitches induced by 5-MeODMT, but this effect was seen at only a single dose of each compound. The benzodiazepine **antagonist** flumazenil did not inhibit the potentiation of head-twitches by diazepam but did itself potentiate head-twitches at 30 mg/kg, consistent with its ability to inhibit adenosine uptake. In contrast, the adenosine uptake inhibitor dipyridamole and the **peripheral-type benzodiazepine receptor antagonist** Ro 5-4864, which also inhibits adenosine uptake, failed to potentiate head-twitches. The adenosine agonists N-6-cyclohexyladenosine, 5'-(N-ethylcarboxamido-adenosine, and (-)-N-6-(R-phenylisopropyl)adenosine were similarly without effect. These results confirm previous findings that the potentiation of head-twitches by benzodiazepines is not mediated via an action at benzodiazepine receptors and suggest that inhibition of adenosine uptake is an important component of the mechanism involved.
...ABSTRACT: addition to their actions at the GABA receptor, benzodiazepines also inhibit adenosine uptake. The adenosine **antagonists** caffeine (0.3-30 mg/kg ip) and 1,3-dipropyl-8-(2-amino-4...

...but this effect was seen at only a single dose of each compound. The benzodiazepine **antagonist** flumazenil did not inhibit the potentiation of head-twitches by diazepam but did itself potentiate...

...its ability to inhibit adenosine uptake. In contrast, the adenosine uptake inhibitor dipyridamole and the **peripheral-type benzodiazepine receptor antagonist** Ro 5-4864, which also inhibits adenosine uptake, failed to potentiate head-twitches. The adenosine...
1993

7/3,K,AB/93 (Item 2 from file: 55)
DIALOG(R)File 55:Biosis Preiviews(R)

09032521 BIOSIS NO.: 199497040891
Identification and characterization of a high-affinity **peripheral-type benzodiazepine receptor** in rabbit urinary bladder smooth muscle.

AUTHOR: Uhlman Eric J; Ruggieri Michael R; Hanno Philip M

AUTHOR ADDRESS: Dep. Urol., Temple Univ. Sch. Med., Philadelphia, PA, USA

JOURNAL: Surgical Forum 44 (0):p764-765 1993
ISSN: 0071-8041
DOCUMENT TYPE: Article
RECORD TYPE: Citation
LANGUAGE: English

Identification and characterization of a high-affinity **peripheral-type benzodiazepine receptor** in rabbit urinary bladder smooth muscle.
MISCELLANEOUS TERMS: ...RECEPTOR **ANTAGONIST**
1993

7/3,K,AB/94 (Item 3 from file: 55)
DIALOG(R)File 55:Biosis Preiviews(R)

8/19/99

08783001 BIOSIS NO.: 199395072352
Diazepam binding inhibitor is a paracrine/autocrine regulator of Leydig cell proliferation and steroidogenesis: Action via **peripheral-type benzodiazepine receptor** and independent mechanisms.

AUTHOR: Garnier Martine; Boujrad Noureddine; Oke Bankole O; Brown A Shane; Riond Joelle; Ferrara Pascual; Shoyab Mohamed; Suarez-Quian Carlos A; Papadopoulos Vassilios(a)
AUTHOR ADDRESS: (a)Dep. Anatomy Cell Biol., Georgetown Univ. Med. Center, 3900 Reservoir Rd. NW, Washington, D.C. 2, USA

JOURNAL: Endocrinology 132 (1):p444-458 1993
ISSN: 0013-7227
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Previous studies demonstrated that the polypeptide diazepam binding inhibitor (DBI) and its **receptor**, the **peripheral-type benzodiazepine receptor** (PBR), are involved in the regulation of steroid biosynthesis and that one site of PBR action resides in mitochondria. In the present investigation, evidence is presented that a functional form of PBR is also present at the cell surface. First, PBR was immunolocalized in the rat testis using biotin-streptavidin peroxidase immunocytochemistry, and results revealed that PBR was present exclusively in the interstitial Leydig cells. Next, the distribution of PBR in MA-10 Leydig cells was further examined using confocal microscopy. MA-10 cells were either fixed and immunostained or fixed/permeabilized and immunostained for PRB followed by generation of confocal microscope optical sections, three-dimensional reconstructions of these sections, and then generation of vertical confocal sections of the three-dimensional reconstruction. In the fixed/unpermeabilized cells, PBR immunostaining at the cell surface was clearly evident, whereas in the fixed/permeabilized cells, intracellular PBR distribution was more robust. These results suggest that the plasma membrane fraction of the receptor could mediate the action of extracellular PBR ligands on Leydig cell function. Next, we examined whether DBI, the naturally occurring PBR ligand, is secreted by testicular cells and whether it could activate the cell surface PBR. Immunolocalization of DBI demonstrated that it was present in both Leydig and Sertoli cells. Further, using an immunoblot assay, we demonstrated that DBI is present in rat testicular interstitial fluid. Metabolic labeling of cultured immature rat Sertoli cells and MA-10 mouse tumor Leydig cells, followed by immunoprecipitation of the secreted proteins with an anti-DBI antiserum, demonstrated that both Leydig and Sertoli cells secrete DBI and could serve as a cell source for

the interstitial fluid DBI. Then, we partially purified the DBI present in conditioned medium and interstitial fluid by reverse phase chromatography and demonstrated it to be bioactive, based on displacement of a radiolabeled benzodiazepine (Ro5-4864)-specific ligand for PBR, pronase treatment of different preparations eliminated all bioactivity. We then examined the effects of DBI on Leydig cell function. DBI added to MA-10 cells affected DNA synthesis and cell growth in a biphasic manner; at low concentrations (1 nM), DBI was mitogenic, increasing (3H)thymidine incorporation and cell numbers by 30-40%, while at high concentrations (1 mu-M), DBI inhibited cell growth (30-40%). Similar effects on cell growth were obtained using the benzodiazepine Ro5-4864. The effects of both DBI and Ro5-4864 were inhibited by the **antagonist** isoquinoline carboxamide PK 11195, suggesting that their actions on cell proliferation were mediated through PBR. DBI directly added to MA-10 or to purified rat Leydig cells also stimulated basal steroid production and potentiated submaxinally hCG-stimulated steroidogenesis (by 2- to 3-fold) in a dose-dependent manner, with an EC-50 of 10 nM. However, the steroidogenic action of DBI was not blocked by PK 11195, but was mimicked by the octadecaneuropeptide (DBI-(33-50)), a peptide with very low affinity for PBR. Because of the widespread occurance of both DBI and PBR in different tissues, we investigated whether DBI may also regulate cell growth and steroid synthesis in other cell models that contain PBR, such as Swiss 3T3 fibroblasts and bovine adrenocortical cells, respectively. The data obtained clearly indicate that activation of PBR by DBI also alters 3T3 fibroblasts cell proliferation, but not effect of DBI on steroid production in adrenocortical cells was observed. These results demonstrate that 1) DBI is secreted by both Leydig and Sertoli cells and is present in the testicular interstitial fluid; 2) DBI, presumably acting via plasma membrane PBR, affects Leydig cell and 3T3 fibroblast DNA synthesis and growth; and 3) DBI, acting via PBR-mediated and/or -independent mechanisms, stimulates Leydig cell steroid production. Thus, we propose that DBI acts as an autocrine/apracrine regulator of Leydig cell function.

...binding inhibitor is a paracrine/autocrine regulator of Leydig cell proliferation and steroidogenesis: Action via **peripheral-type benzodiazepine receptor** and independent mechanisms.

ABSTRACT: Previous studies demonstrated that the polypeptide diazepam binding inhibitor (DBI) and its **receptor**, the **peripheral-type benzodiazepine receptor** (PBR), are involved in the regulation of steroid biosynthesis and that one site of PBR...

...benzodiazepine Ro5-4864. The effects of both DBI and Ro5-4864 were inhibited by the **antagonist** isoquinoline carboxamide PK 11195, suggesting that their actions on cell proliferation were mediated through PBR...

1993

? ds

Set	Items	Description
S1	574	PERIPHERAL(W)TYPE(5N)BENZODIAZEPINE(5N)RECEPTOR? ?
S2	145689	ANTAGONIST
S3	105	S1 AND S2
S4	437722	ANTAGONIST? ?
S5	137	S1 AND S4
S6	134	S5 AND PY<=1998
S7	94	RD (unique items)

? t s7/3,k,ab/1-10

8/19/99

7/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09861101 99057292

Effect of benzodiazepines and neurosteroids on ammonia-induced swelling in cultured astrocytes.

Bender AS; Norenberg MD
Veterans Administration Medical Center, Department of Pathology, University of Miami School of Medicine, Florida 33101, USA.

J Neurosci Res (UNITED STATES) Dec 1 **1998**, 54 (5) p673-80,
ISSN 0360-4012 Journal Code: KAC
Contract/Grant No.: NS30291, NS, NINDS; DK38153, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Astroglial swelling occurs in acute hyperammonemic states, including acute hepatic encephalopathy. In these conditions, the **peripheral-type benzodiazepine receptor** (PBR), a **receptor** associated with neurosteroidogenesis, is up-regulated. This study examined the potential involvement of PBRs and neurosteroids in ammonia-induced astrocyte swelling in culture. At low micromolar concentrations, the PBR **antagonist** PK 11195, atrial natriuretic peptide, and protoporhyrin IX, which are known to interact with the PBR, attenuated (16-100%) the effects of ammonia, whereas the PBR agonists Ro5-4864, diazepam binding inhibitor (DBI51-70), and octadecaneuropeptide exacerbated (10-15%) the effects of ammonia. At micromolar concentrations, diazepam, which interacts with both the PBR and the central-type benzodiazepine receptor (CBR), increased swelling by 11%, whereas flumazenil, a CBR **antagonist**, had no effect. However, at 100 nM diazepam and flumazenil abrogated ammonia-induced swelling. The neurosteroids dehydroepiandrosterone sulfate, tetrahydroprogesterone, pregnenolone sulfate, and tetrahydrodeoxycorticosterone (THDOC), products of PBR stimulation, at micromolar concentrations significantly enhanced (70%) ammonia-induced swelling. However, at nanomolar concentrations, these neurosteroids, with exception of THDOC, blocked ammonia-induced swelling. We conclude that neurosteroids and agents that interact with the PBR influence ammonia-induced swelling. These agents may represent novel therapies for acute hyperammonemic syndromes and other conditions associated with brain edema and astrocyte swelling.

Dec 1 **1998**,
Astroglial swelling occurs in acute hyperammonemic states, including acute hepatic encephalopathy. In these conditions, the **peripheral-type benzodiazepine receptor** (PBR), a **receptor** associated with neurosteroidogenesis, is up-regulated. This study examined the potential involvement of PBRs and neurosteroids in ammonia-induced

astrocyte swelling in culture. At low micromolar concentrations, the PBR **antagonist** PK 11195, atrial natriuretic peptide, and protoporhyrin IX, which are known to interact with the...

...and the central-type benzodiazepine receptor (CBR), increased swelling by 11%, whereas flumazenil, a CBR **antagonist**, had no effect. However, at 100 nM diazepam and flumazenil abrogated ammonia-induced swelling. The ...

...; PD; Flumazenil--Pharmacology--PD; Isoquinolines--Pharmacology--PD; Nerve Tissue Proteins--Agonists--AG; Nerve Tissue Proteins--**Antagonists** and Inhibitors--AI; Nerve Tissue Proteins--Biosynthesis --BI; Neuropeptides--Pharmacology--PD; Peptide Fragments--Pharmacology--PD ...

...PD; Pregnenolone--Pharmacology--PD; Protoporphyrins--Pharmacology--PD; Receptors, GABA-A--Agonists--AG; Receptors, GABA-A--**Antagonists** and Inhibitors--AI; Receptors, GABA-A--Biosynthesis--BI; Up-Regulation (Physiology)--Drug Effects--DE

7/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09808795 99027577

Diazepam potentiates the positive inotropic effects of histamine and forskolin in guinea-pig papillary muscles.

Hara Y; Kaigo H; Minami I; Watanabe H; Gomi H; Nishimura H; Chugun A; Kondo H

Department of Veterinary Pharmacology, School of Veterinary Medicine and Animal Sciences, Kitasato University, Aomori, Japan.

J Vet Pharmacol Ther (ENGLAND) Oct **1998**, 21 (5) p375-9, ISSN 0140-7783 Journal Code: KCP

Languages: ENGLISH

Document type: JOURNAL ARTICLE

There have been diverse reports on the effects of diazepam on cardiac contractility. The purpose of this study was to examine whether diazepam modifies the inotropic response elicited by histamine on an isolated guinea-pig papillary muscle. The responses of electrically driven papillary muscle to histamine and cyclic AMP-related inotropic agents were recorded in the absence and in the presence of diazepam. Histamine and forskolin, which directly stimulate adenylate cyclase, significantly increased the contractile force in the papillary muscle in a concentration-dependent manner. A histaminergic H2-receptor **antagonist**, cimetidine, but not a H1-receptor **antagonist** , diphenhydramine, at 10 microM produced a rightward shift in the concentration-response curve for histamine. Diazepam (10 microM) shifted the concentration-response curve for histamine and forskolin to the left by 1.8 and 1.6 times, respectively. Neither a central type (fulmazenil) nor a **peripheral type** (PK11195) of **benzodiazepine receptor antagonist** modified the effect of diazepam on the histaminergic-evoked contraction. Phosphodiesterase blockade by 3-isobutyl-1-methylxanthine shifted the concentration-dependent curve for histamine to the left. A combination of 3-isobutyl-1-methylxanthine also produced a leftward shift of the curve. However, there was no significant difference between the 3-isobutyl-1-methylxanthine only group and the combination group. These results indicate that diazepam potentiates the positive inotropic effect produced by histamine, probably mediated via an increase in cyclic AMP levels induced by histamine.

Oct **1998**,

...contractile force in the papillary muscle in a concentration-dependent manner. A histaminergic H2-receptor **antagonist**, cimetidine, but not a H1-receptor **antagonist** , diphenhydramine, at 10 microM produced a rightward shift in the concentration-response curve for histamine...

... by 1.8 and 1.6 times, respectively. Neither a central type (fulmazenil) nor a **peripheral type** (PK11195) of **benzodiazepine receptor antagonist** modified the effect of diazepam on the histaminergic-evoked contraction. Phosphodiesterase blockade by 3-isobutyl ...

7/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09798445 99049236
Inhibitory regulation of amylase release in rat parotid acinar cells by benzodiazepine receptors.
Okubo M; Kawaguchi M
Department of Pharmacology and Oral Health Science Center, Tokyo Dental College, Chiba, Japan.
Eur J Pharmacol (NETHERLANDS) Oct 23 **1998**, 359 (2-3) p243-9, ISSN 0014-2999 Journal Code: EN6
Languages: ENGLISH
Document type: JOURNAL ARTICLE
This study examined the influence of benzodiazepine receptors on amylase release from rat parotid acinar cells. Diazepam (10(-8)-10(-6) M), which is a potent agonist of both central- and **peripheral-type benzodiazepine receptors** , dose dependently decreased amylase release induced by isoprenaline and carbachol, which are beta-adrenoceptor and muscarinic receptor agonists, respectively. The maximum inhibitory response was obtained with 10(-6) M diazepam: amylase release was decreased to 57% (isoprenaline) and 39% (carbachol) of maximal levels, while these responses were completely inhibited by propranolol and atropine, respectively. Clonazepam and 7-chloro-1,3-dihydro-1-methyl-5-p-chlorophenyl)-2H-1,4-benzodiazepine-2- one (Ro 5-4864), which are selective agonists of central- and **peripheral-type benzodiazepine receptors** , respectively, also produced a significant and dose-dependent decrease in isoprenaline-induced amylase release. The inhibitory potency was diazepam > clonazepam > Ro 5-4864. Flumazenil and 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide (PK 11195), which are selective **antagonists** of central- and **peripheral-type benzodiazepine receptors** , respectively, dose dependently blocked the inhibition of isoprenaline-induced amylase release by diazepam. At a concentration of 10(-5) M, flumazenil and PK 11195 restored amylase release to approximately 75% of that in the presence of isoprenaline alone. The combination of both **antagonists** completely prevented the inhibition by diazepam. Similarly, the inhibitory responses of clonazepam and Ro 5-4864 were completely blocked by flumazenil and PK 11195, respectively. These results suggest that, in rat parotid acinar cells, benzodiazepines inhibit beta-adrenoceptor and muscarinic **receptor**-stimulated amylase release and that both central- and **peripheral-type benzodiazepine receptors** contribute to this inhibitory regulation.

Oct 23 **1998**,
...Diazepam (10(-8)-10(-6) M), which is a potent agonist of both central- and **peripheral-type benzodiazepine receptors**, dose dependently decreased amylase release induced by isoprenaline and carbachol, which are beta-adrenoceptor and...

... 1,4-benzodiazepine-2- one (Ro 5-4864), which are selective agonists of central- and **peripheral-type benzodiazepine receptors** , respectively, also produced a significant and dose-dependent decrease in isoprenaline-induced amylase release. The...

... 2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide (PK 11195), which are selective **antagonists** of central- and

peripheral-type benzodiazepine receptors, respectively, dose dependently blocked the inhibition of isoprenaline-induced amylase release by diazepam. At a...

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...These results suggest that, in rat parotid acinar cells, benzodiazepines inhibit beta-adrenoceptor and muscarinic **receptor**-stimulated amylase release and that both central- and **peripheral-type benzodiazepine receptors** contribute to this inhibitory regulation.
; Adrenergic beta-Agonists--Pharmacology--PD; Adrenergic beta-**Antagonists**--Pharmacology--PD; Amylases--Drug Effects--DE; Atropine --Pharmacology--PD; Benzodiazepinones--Pharmacology--PD; Carbachol --Pharmacology--PD...

...Flumazenil--Pharmacology--PD; GABA Modulators--Pharmacology--PD; Isoproterenol--Pharmacology--PD; Muscarinic Agonists--Pharmacology--PD; Muscarinic **Antagonists**--Pharmacology--PD; Parotid Gland--Cytology--CY ; Parotid Gland--Drug Effects--DE; Propranolol--Pharmacology--PD; Rats; Rats, Wistar; Receptors, GABA-A--Agonists--AG; Receptors, GABA-A--**Antagonists** and Inhibitors--AI
Chemical Name: Amylases; (Adrenergic beta-Agonists; (Adrenergic beta-**Antagonists**; (Benzodiazepinones; (GABA Modulators; (Muscarinic Agonists; (Muscarinic **Antagonists**; (Receptors, GABA-A; (Ro 5-4864; (Clonazepam; (Diazepam; (Atropine; (Carbachol; (Propranolol; (Isoprotereno l; (Flumazenil

7/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09680920 97094315
In vivo regulation of **peripheral-type benzodiazepine receptor** and glucocorticoid synthesis by Ginkgo biloba extract EGb 761 and isolated ginkgolides.
Amri H; Ogwuegbu SO; Boujrad N; Drieu K; Papadopoulos V
Department of Cell Biology, Georgetown University Medical Center, Washington, District of Columbia 20007, USA.
Endocrinology (UNITED STATES) Dec **1996**, 137 (12) p5707-18, ISSN 0013-7227 Journal Code: EGZ
Contract/Grant No.: ES-07747, ES, NIEHS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Glucocorticoid excess has broad pathogenic potential including neurotoxicity, neuroendangerment, and immunosuppression. Glucocorticoid synthesis is regulated by ACTH, which acts by accelerating the transport of the precursor cholesterol to the mitochondria where steroidogenesis begins. Ginkgo biloba is one of the most ancient trees, and extracts from its leaves have been used in traditional medicine. A standardized extract of Ginkgo biloba leaves, termed EGb 761 (EGb), has been shown to have neuroprotective and antistress effects. In vivo treatment of rats with EGb, and its bioactive components ginkgolide A and B, specifically reduces the ligand binding capacity, protein, and messenger RNA expression of the adrenocortical mitochondrial **peripheral-type benzodiazepine%
%% receptor (PBR), a key element in the regulation of cholesterol transport, resulting in decreased corticosteroid synthesis. As expected, the ginkgolide-induced decrease in glucocorticoid levels resulted in increased ACTH release, which in turn induced the expression of the steroidogenic acute regulatory protein. Because ginkgolides reduced the adrenal PBR expression and corticosterone synthesis despite the presence of high levels of steroidogenic acute regulatory protein, these data**

demonstrate that PBR is indispensable for normal adrenal function. In addition, these results suggest that manipulation of PBR expression could control circulating glucocorticoid levels, and that the antistress and neuroprotective effects of EGb are caused by to its effect on glucocorticoid biosynthesis.

In vivo regulation of peripheral-type benzodiazepine receptor and glucocorticoid synthesis by Ginkgo biloba extract EGb 761 and isolated ginkgolides.
Dec 1996,
... specifically reduces the ligand binding capacity, protein, and messenger RNA expression of the adrenocortical mitochondrial peripheral-type benzodiazepine receptor (PBR), a key element in the regulation of cholesterol transport, resulting in decreased corticosteroid synthesis...
...; Immunoblotting; Immunohistochemistry; Isoquinolines--Metabolism--ME; Mitochondria--Metabolism--ME; Rats; Rats, Sprague-Dawley; Receptors, GABA-A --Antagonists and Inhibitors--AI; Receptors, GABA-A--Genetics--GE; RNA, Messenger--Antagonists and Inhibitors--AI; RNA, Messenger --Metabolism--ME

7/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09576491 98267064
U-83836E prevents kainic acid-induced neuronal damage.
Camins A; Gabriel C; Aguirre L; Sureda FX; Pubill D; Pallas M; Escubedo E ; Camarasa J
Unitat de Farmacologia i Farmacognosia, Facultat de Farmacia, Universitat de Barcelona, Spain.
Naunyn Schmiedebergs Arch Pharmacol (GERMANY) Apr 1998, 357 (4) p413-8, ISSN 0028-1298 Journal Code: NTQ
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The effect of kainic acid (KA) on mitochondrial membrane potential (MMP) and reactive-oxygen species (ROS) production was studied in dissociated cerebellar granule cells from rat pups. KA induced a maximum increase of 361%+/-35% in ROS production. The lazaroid compound U-83836E (at concentrations ranging from 10(-9) to 5x10(-6) M) completely inhibited this increase, with an IC50 value of 3.02+/-1.08x10(-7) M. KA also decreased the mitochondrial membrane potential (MMP), with a maximum decrease of about 30%. Absence of Na+ in the incubation medium did not significantly alter the effect of KA on MMP. As expected, the AMPA/kainate receptor antagonist NBQX inhibited the effects of KA on MMP with an IC50 value of 1.1+/-0.8 microM. However, the lazaroid U-83836E, indomethacin, nor-dihydroguaiaretic acid and L-nitroarginine all failed to inhibit the KA-induced decrease in the MMP. Finally, to assess the neuroprotective effect of U-83836E on KA-induced neurotoxicity in vivo, the increase in the peripheral-type benzodiazepine receptor density in rat hippocampus was measured. Treatment with KA increased the Bmax to 1341+/-192 fmol mg(-1). When U-83836E was coadministered with KA, the Bmax was reduced to 765+/-122 fmol mg(-1), which was not significantly different from the Bmax obtained from untreated rats (Bmax: 518+/-33 fmol mg(-1)). We conclude that treatment with the lazaroid U-83836E might be a suitable therapeutic strategy in neurodegenerative disorders.

Apr 1998,
... not significantly alter the effect of KA on MMP. As expected, the AMPA/kainate receptor antagonist NBQX inhibited the effects of KA on MMP with an IC50 value of 1.1...

... neuroprotective effect of U-83836E on KA-induced neurotoxicity in vivo, the increase in the peripheral-type benzodiazepine

receptor density in rat hippocampus was measured. Treatment with KA increased the Bmax to 1341+/-192...

7/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09502464 98218812
Functional and biochemical evidence for diazepam as a cyclic nucleotide phosphodiesterase type 4 inhibitor.
Collado MC; Beleta J; Martinez E; Miralpeix M; Domenech T; Palacios JM; Hernandez J
Department of Pharmacology, Medical School, Murcia, Spain.
Br J Pharmacol (ENGLAND) Mar 1998, 123 (6) p1047-54, ISSN 0007-1188 Journal Code: B00
Languages: ENGLISH
Document type: JOURNAL ARTICLE
1. The responses of the electrically-driven right ventricle strip of the guinea-pig heart to diazepam were recorded in the absence and in the presence of different selective cyclic nucleotide phosphodiesterase (PDE) inhibitors. 2. Diazepam, at concentrations ranging from 1 microM to 100 microM, was devoid of effect on the contractile force in this preparation. 3. Conversely, diazepam (5 microM-100 microM) produced a consistent positive inotropic response in the presence of a concentration (1 microM), that was without effect in the absence of diazepam, of either of the selective PDE 3 inhibitors milrinone or SK&F 94120, but not in the presence of the selective PDE 4 inhibitor rolipram. 4. This effect of diazepam was not gamma-aminobutyric acid (GABA)-dependent, since it was neither mimicked nor potentiated by GABA, and was not affected by either a high concentration (5 microM) of the antagonists of the benzodiazepine/GABA/channel chloride receptor complex, picrotoxin, flumazenil and beta-carboline-3-carboxylic acid methyl ester (betaCCMe), or by the inverse agonists, beta-carboline-3-carboxylic acid N-methylamide (betaCCMa) and methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM, 0.1 microM). Furthermore, a specific antagonist of the peripheral benzodiazepine receptors, PK 11195 (5 microM), did not influence the effect of diazepam. 5. Biochemical studies with isolated PDEs, confirmed that diazepam selectively inhibits type 4 PDE from guinea-pig right ventricle rather than the other PDEs present in that tissue. The compound inhibited this enzyme in a non-competitive manner. Diazepam was also able to inhibit PDE 5, the cyclic GMP specific PDE absent from cardiac muscle, with a potency close to that shown for PDE 4. 6. Diazepam displaced the selective type 4 PDE inhibitor, rolipram from its high affinity binding site in rat brain cortex membranes, and also potentiated the rise in cyclic AMP levels induced by isoprenaline in guinea-pig eosinophils, where only type 4 PDE is present. 7. The PDE inhibitory properties of diazepam were shared, although with lower potency, by other structurally-related benzodiazepines, that also displaced [3H]-rolipram from its high affinity binding site. The order of potency found for these compounds in these assays was not related to their potencies as modulators of the GABA receptor through its benzodiazepine binding site. 8. The pharmacological and biochemical data presented in this study indicate that diazepam behaves as a selective type 4 PDE inhibitor in cardiac tissue and this effect seems neither to be mediated by the benzodiazepine/GABA/channel chloride receptor complex nor by peripheral type benzodiazepine receptors.

Mar 1998,
... by GABA, and was not affected by either a high concentration (5 microM) of the antagonists of the benzodiazepine/GABA/channel chloride receptor complex, picrotoxin, flumazenil and beta-carboline-3-carboxylic...

... 7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM, 0.1 microM).

Furthermore, a specific antagonist of the peripheral benzodiazepine receptors, PK 11195 (5 microM), did not influence the effect of...

... PDE inhibitor in cardiac tissue and this effect seems neither to be mediated by the benzodiazepine/GABA/channel chloride receptor complex nor by peripheral type benzodiazepine receptors.
...; Eosinophils--Metabolism--ME; Guinea Pigs; GABA--Metabolism--ME; GABA--Physiology--PH; Heart--Physiology--PH; Isoenzymes--Antagonists and Inhibitors--AI; Isoenzymes--Metabolism--ME; Isoproterenol --Pharmacology--PD; Myocardial Contraction--Drug Effects--DE; Pyrrolidinones...

7/3,K,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09450641 98157936
Effects of peripheral-type benzodiazepine receptor antisense knockout on MA-10 Leydig cell proliferation and steroidogenesis.
Kelly-Hershkovitz E; Weizman R; Spanier I; Leschiner S; Lahav M; Weisinger G; Gavish M
Department of Pharmacology, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, 31096 Haifa, Israel.
J Biol Chem (UNITED STATES) Mar 6 1998, 273 (10) p5478-83,
ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The peripheral-type benzodiazepine receptor (PBR) is not only widely expressed throughout the body, but it is also genetically conserved from bacteria to humans. Many functions have been attributed to it, but its primary role remains a puzzle. In the current study, we stably transfected cultures of MA-10 Leydig cells with either control or 18-kDa PBR antisense knockout plasmids. The antisense knockout vector was driven by the human enkephalin promoter, which contains two cAMP response elements, such that cAMP treatment of transfected cells could superinduce 18-kDa PBR antisense RNA transcription and, hence, down-regulate endogenous 18-kDa PBR mRNA levels. Control and knockout MA-10 cell lines were then compared at the level of receptor binding, thymidine incorporation, and steroid biosynthesis. Eighteen-kilodalton PBR knockout reduced the maximal binding capacity of tritium-labeled PBR ligands, and the affinity of receptors to the ligands remained unaltered. Additionally, 24-h accumulation of progesterone was lower in the knockout cells. Exposure of the two cell types to 8-bromo-cAMP resulted in a robust increase in steroid production. However, a complex pattern of steroid accumulation was observed, in which further progestin metabolism was indicated. The later decline in accumulated progesterone as well as the synthesis of androstenedione were different in the two cell types. At the level of cell proliferation, reduction of 18-kDa PBR mRNA showed no effect. Thus, we conclude that the 18-kDa PBR may have a more important role in steroidogenesis than in proliferation in this Leydig cell line.

Effects of peripheral-type benzodiazepine receptor antisense knockout on MA-10 Leydig cell proliferation and steroidogenesis.
Mar 6 1998,
The peripheral-type benzodiazepine receptor (PBR) is not only widely expressed throughout the body, but it is also genetically conserved...
Descriptors: DNA, Antisense--Pharmacology--PD; *Leydig Cells--Metabolism --ME; *Receptors, GABA-A--Antagonists and Inhibitors--AI

7/3,K,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09447010 98174266
Effect of the triakontatetraneuropeptide (TTN) on corticosteroid secretion by the frog adrenal gland.
Lesouhaitier O; Feuilloley M; Vaudry H
European Institute for Peptide Research (IFRMP No. 23), INSERM U 413, UA CNRS, University of Rouen, Mont-Saint-Aignan, France.
J Mol Endocrinol (ENGLAND) Feb 1998, 20 (1) p45-53, ISSN 0952-5041 Journal Code: AEG
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Diazepam-binding inhibitor (DBI) was initially isolated from the rat brain as a result of its ability to compete with benzodiazepines for their receptors . Immunohistochemical studies have recently shown the presence of peripheral-type benzodiazepine receptor (PBR)- and DBI-like immunoreactivity in the frog adrenal gland. The aim of the present study was to investigate the effect of two biologically active DBI-derived peptides, the triakontatetraneuropeptide [TTN; DBI(17-50)] and the octadecaneuropeptide [ODN; DBI(33-50)], on corticosteroid secretion by frog adrenocortical cells. Exposure of frog adrenal explants to graded concentrations of TTN (3.16 x 10(-8) to 3.16 x 10(-6) M) induced a dose-related increase in corticosterone and aldosterone secretion. In contrast, ODN did not modify corticosteroid output. When repeated pulses of TTN (10(-6) M) were administered at 2-h intervals, the response of the adrenal explants to the second dose of TTN was markedly reduced, suggesting the existence of a desensitization phenomenon. Exposure of dispersed adrenal cells to TTN also induced a marked stimulation of corticosteroid secretion, indicating that TTN acts directly on adrenocortical cells. The central-type benzodiazepine receptor (CBR) agonist, clonazepam, did not stimulate corticosteroid secretion and the CBR antagonist , flumazenil, did not block the stimulatory action of TTN. Similarly, the PBR agonist, Ro5-4864, did not mimic the stimulatory effect of TTN and the PBR antagonist , flunitrazepam, did not affect the stimulatory action of TTN. The present study provides the first evidence for a stimulatory effect of TTN on intact adrenocortical cells. The receptor mediating the corticotropic action of TTN is not related to central- or peripheral-type benzodiazepine receptors. Our data suggest that TTN, released by chromaffin cells, may act as a paracrine factor regulating the activity of adrenocortical cells.

Feb 1998,
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... cells. The receptor mediating the corticotropic action of TTN is not related to central- or peripheral-type benzodiazepine receptors . Our data suggest that TTN, released by chromaffin cells, may act as a paracrine factor...

7/3,K,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09421685 98122094

The endozepine triakontatetraneuropeptide diazepam-binding inhibitor [17-50] stimulates neurosteroid biosynthesis in the frog hypothalamus.

Do-Rego JL; Mensah-Nyagan AG; Feuilloley M; Ferrara P; Pelletier G; Vaudry H

European Institute for Peptide Research (IFRMP no 23), Laboratory of Cellular and Molecular Neuroendocrinology, INSERM U 413, UA CNRS, University of Rouen, Mont-Saint-Aignan, France.

Neuroscience (UNITED STATES) Mar 1998, 83 (2) p555-70, ISSN 0306-4522 Journal Code: NZR

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Neurons and glial cells are capable of synthesizing various bioactive steroids, but the neuronal mechanisms controlling neurosteroid-secreting cells are poorly understood. In the present study, we have investigated the possible effect of an endogenous ligand of benzodiazepine receptors, the triakontatetraneuropeptide [17-50] (TTN), on steroid biosynthesis in the frog hypothalamus. Immunohistochemical studies revealed that most hypothalamic neurons expressing 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase also contained peripheral-type benzodiazepine receptor -like immunoreactivity. Confocal laser scanning microscopic analysis revealed that the peripheral-type benzodiazepine receptor -immunoreactive material was located both in the cytoplasm and at the periphery of the cell bodies. By using the pulse-chase technique, TTN was found to stimulate the conversion of [3H]pregnenolone into various steroids, including 17-hydroxypregnenolone, 5 alpha-dihydrotestosterone and 17-hydroxyprogesterone, in a dose-dependent manner. The peripheral-type benzodiazepine receptor agonist Ro5-4864 mimicked the stimulatory effect of TTN on the formation of neurosteroids. The peripheral-type benzodiazepine receptor antagonist PK11195 significantly reduced the effect of TTN on neurosteroid synthesis, while the central-type benzodiazepine receptor antagonist flumazenil did not affect the formation of neurosteroids evoked by TTN. These data indicate that TTN stimulates the biosynthesis of 3-keto-17 alpha-hydroxysteroids in frog hypothalamic neurons through activation of peripheral-type benzodiazepine receptors likely located at the plasma membrane level.

Mar 1998,

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...the biosynthesis of 3-keto-17 alpha-hydroxysteroids in frog hypothalamic neurons through activation of peripheral-type benzodiazepine receptors likely located at the plasma membrane level.

...; DE; Immunohistochemistry; Peripheral Nerves--Enzymology--EN;

Peripheral Nerves--Metabolism--ME; Rana ridibunda; Receptors, GABA-A--Antagonists and Inhibitors--AI; Receptors, GABA-A--Metabolism--ME; Stimulation, Chemical; 3-Hydroxysteroid Dehydrogenases--Metabolism--ME

7/3,K,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09384087 97469139
Effects of midazolam on glycemia and serum lipids in rats.
Cuparencu B; Horak J; Horak A; Lenghel A
Department of Pharmacology, University of Oradea, Romania.
Acta Physiol Hung (HUNGARY) 1996, 84 (4) p389-98, ISSN 0231-424X Journal Code: 1RS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Midazolam administered ip. in albino rats (each group consisted from 10 animals rendered hyperdyslipidemic by the administration of Triton WR-1339) induced at most doses a significant reduction of glycemia ($p < 0.001$). However, the reduction of blood glucose level was outside of the dangerous level. Midazolam elicited also very significant decrease of the elevated serum lipids ($p < 0.001$). The pharmacological analysis of these phenomena by using the peripheral type benzodiazepine (BZD) receptors antagonist PK 11105, the central BZD receptor antagonist flumazenil and the purinergic P1 receptors antagonist aminophylline has shown that the effects on serum lipids were due, very probably to the stimulation of the peripheral type BZD receptors. Aminophylline seems to have the property to block the peripheral type BZD receptors. The effects on blood glucose level were very variable.
1996,
... elevated serum lipids ($p < 0.001$). The pharmacological analysis of these phenomena by using the peripheral type benzodiazepine (BZD) receptors antagonist PK 11105, the central BZD receptor antagonist flumazenil and the purinergic P1 receptors antagonist aminophylline has shown that the effects on serum lipids were due, very probably to the...
; Aminophylline--Pharmacology--PD; Anti-Anxiety Agents, Benzodiazepine--Antagonists and Inhibitors--AI; Antilipemic Agents--Pharmacology--PD; Drug Interactions; Flumazenil--Pharmacology--PD; GABA Modulators--Pharmacology--PD; Isoquinolines--Pharmacology--PD; Midazolam--Antagonists and Inhibitors--AI; Phosphodiesterase Inhibitors--Pharmacology--PD; Procetofen--Pharmacology--PD; Rats; Rats, Wistar

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    $0.00  Estimated total session cost   0.148 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-1999/Oct W1
         (c) format only 1999 Dialog Corporation
*File 155: reloaded, note accession numbers changed.
  File 434:SciSearch(R) Cited Ref Sci  1974-1989/Dec
         (c) 1998 Inst for Sci Info
  File  55:Biosis Preiviews(R)  1993-1999/Jul W4
         (c) 1999 BIOSIS
*File  55: File is reloaded. Accession number changed.

      Set  Items  Description
      ---  -----  -----------
? s peripheral(w)type(5n)benzodiazepine(5n)receptor? ?

          319828  PERIPHERAL
          853185  TYPE
           31270  BENZODIAZEPINE
          889466  RECEPTOR? ?
      S1     574  PERIPHERAL(W)TYPE(5N)BENZODIAZEPINE(5N)RECEPTOR? ?
? s antagonist? ?

      S2  437722  ANTAGONIST? ?
? s s1 and s2

             574  S1
          437722  S2
      S3     137  S1 AND S2
? s antibod?

      S4  805198  ANTIBOD?
? s s3 and s4

             137  S3
          805198  S4
      S5       6  S3 AND S4
? rd

...completed examining records
      S6       6  RD (unique items)
? t s6/3,k,ab/1-6


 6/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

09421685   98122094
  The  endozepine triakontatetraneuropeptide diazepam-binding inhibitor
[17-50] stimulates neurosteroid biosynthesis in the frog hypothalamus.
  Do-Rego JL; Mensah-Nyagan AG; Feuilloley M; Ferrara P; Pelletier G;
Vaudry H
  European Institute for Peptide Research (IFRMP no 23), Laboratory of
Cellular and Molecular Neuroendocrinology, INSERM U 413, UA CNRS,
University of Rouen, Mont-Saint-Aignan, France.
  Neuroscience (UNITED STATES)  Mar 1998,  83 (2) p555-70,  ISSN 0306-4522
Journal Code: NZR
  Languages: ENGLISH
```

Document type: JOURNAL ARTICLE

Neurons and glial cells are capable of synthesizing various bioactive steroids, but the neuronal mechanisms controlling neurosteroid-secreting cells are poorly understood. In the present study, we have investigated the possible effect of an endogenous ligand of benzodiazepine receptors, the triakontatetraneuropeptide [17-50] (TTN), on steroid biosynthesis in the frog hypothalamus. Immunohistochemical studies revealed that most hypothalamic neurons expressing 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase also contained **peripheral-type benzodiazepine receptor** -like immunoreactivity. Confocal laser scanning microscopic analysis revealed that the **peripheral-type benzodiazepine receptor** -immunoreactive material was located both in the cytoplasm and at the periphery of the cell bodies. By using the pulse-chase technique, TTN was found to stimulate the conversion of [3H]pregnenolone into various steroids, including 17-hydroxypregnenolone, 5 alpha-dihydrotestosterone and 17-hydroxyprogesterone, in a dose-dependent manner. The **peripheral-type benzodiazepine receptor** agonist Ro5-4864 mimicked the stimulatory effect of TTN on the formation of neurosteroids. The **peripheral-type benzodiazepine receptor antagonist** PK11195 significantly reduced the effect of TTN on neurosteroid synthesis, while the central-type benzodiazepine receptor **antagonist** flumazenil did not affect the formation of neurosteroids evoked by TTN. These data indicate that TTN stimulates the biosynthesis of 3-keto-17 alpha-hydroxysteroids in frog hypothalamic neurons through activation of **peripheral-type benzodiazepine receptors** likely located at the plasma membrane level.

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... including 17-hydroxypregnenolone, 5 alpha-dihydrotestosterone and 17-hydroxyprogesterone, in a dose-dependent manner. The **peripheral-type benzodiazepine receptor** agonist Ro5-4864 mimicked the stimulatory effect of TTN on the formation of neurosteroids. The **peripheral-type benzodiazepine receptor antagoni st** PK11195 significantly reduced the effect of TTN on neurosteroid synthesis, while the central-type benzodiazepine receptor **antagonist** flumazenil did not affect the formation of neurosteroids evoked by TTN. These data indicate that...

...the biosynthesis of 3-keto-17 alpha-hydroxysteroids in frog hypothalamic neurons through activation of **peripheral-type benzodiazepine receptors** likely located at the plasma membrane level.

; Benzodiazepinones--Pharmacology--PD; Chromatography, High Pressure Liquid; Convulsants--Pharmacology--PD; Fluorescent **Antibody** Technique, Direct; Hypothalamus--Drug Effects--DE; Immunohistochemistry; Peripheral Nerves--Enzymology--EN; Peripheral Nerves--Metabolism--ME; Rana ridibunda; Receptors, GABA-A--**Antagonists** and Inhibitors--AI; Receptors, GABA-A--Metabolism--ME; Stimulation, Chemical; 3-Hydroxysteroid Dehydrogenases--Metabolism--ME

6/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09127611 97341652

Significant inhibition of spontaneous IgA secretion by selective

peripheral-type benzodiazepine receptor ligands.
Bessler H; Caspi B; Gavish M; Rehavi M; Weizman A
Hematology and Immunology Research Laboratory, Rabin Medical Center, Petah Tiqva, Israel.
Clin Neuropharmacol (UNITED STATES) Jun 1997, 20 (3) p215-23, ISSN 0362-5664 Journal Code: CNK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The in vitro effect of benzodiazepine (BZ) receptor ligands on the secretion of immunoglobulin isotypes IgM, IgG, and IgA by human peripheral blood mononuclear cells (PBMCs) was examined. It was found that the specific peripheral-type BZ receptor (PBR) ligands (Ro5-4864 and PK 11195) inhibit the spontaneous secretion of IgA by human PBMCs in a dose-dependent manner, in the micromolar range. The decreased secretion of IgG and IgM induced by these ligands did not reach significant levels. The mixed BZ ligands (diazepam and flunitrazepam) had no consistent or significant effect on the production of the three immunoglobulin isotypes tested in the current study. The central-type ligand (clonazepam) did not affect IgM, IgG, or IgA secretion. The significant inhibitory effect of PBR ligands was confined to the spontaneous secretion of IgA by human PBMCs, and no such effect was detected in cells stimulated by pokeweed mitogen to produce immunoglobulins. It seems that PBR ligands are capable of suppressing spontaneous IgA secretion, but fail to affect the augmented production induced by mitogen.

Significant inhibition of spontaneous IgA secretion by selective **peripheral-type benzodiazepine receptor** ligands.
Descriptors: Anti-Anxiety Agents, Benzodiazepine--Pharmacology--PD; * **Antibody**-Producing Cells--Drug Effects--DE; *B-Lymphocytes --Drug Effects--DE; *IgA--Metabolism--ME; *IgG--Metabolism--ME; *IgM--Metabolism --ME; *Receptors, GABA-A--**Antagonists** and Inhibitors--AI

6/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06167509 87022287
Purification of an endogenous benzodiazepine-like substance from the mammalian brain.
De Blas AL; Sangameswaran L
Adv Biochem Psychopharmacol (UNITED STATES) 1986, 42 p57-67, ISSN 0065-2229 Journal Code: 2I8
Contract/Grant No.: NS17708, NS, NINDS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
An anti-benzodiazepine monoclonal **antibody** has been used to demonstrate the existence of benzodiazepine-like molecules in the brain and for the purification of these molecules. Immunocytochemical experiments show that these molecules are neuronal and not glial and that they are ubiquitously distributed throughout the brain. Immunoblots indicate the presence of benzodiazepine-like epitopes in several brain peptides. An endogenous substance that binds to the central-type benzodiazepine receptor with agonist properties has been purified to homogeneity from the bovine brain. The purification consisted on immunoaffinity chromatography on immobilized monoclonal anti-benzodiazepine **antibody** followed by gel filtration on Sephadex G-25 and two reverse phase HPLCs. The purified substance has a small molecular weight and its activity is protease resistant. The endogenous substance blocks the binding of agonists, inverse agonists and **antagonists** to the central-type benzodiazepine receptor but it does not inhibit the binding of Ro5-4864 to the "**peripheral-type" benzodiazepine receptor** . The neurotransmitter gamma-amino-butyric acid increases the affinity of the benzodiazepine receptor for the purified substance. Thus this benzodiazepine-like substance is different from the endogenous benzodiazepine receptor ligands

reported by others.

An anti-benzodiazepine monoclonal **antibody** has been used to demonstrate the existence of benzodiazepine-like molecules in the brain and ...

... from the bovine brain. The purification consisted on immunoaffinity chromatography on immobilized monoclonal anti-benzodiazepine **antibody** followed by gel filtration on Sephadex G-25 and two reverse phase HPLCs. The purified...

... activity is protease resistant. The endogenous substance blocks the binding of agonists, inverse agonists and **antagonists** to the central-type benzodiazepine receptor but it does not inhibit the binding of Ro5-4864 to the **"peripheral-type" benzodiazepine receptor**. The neurotransmitter gamma-amino-butyric acid increases the affinity of the benzodiazepine receptor for the...

; **Antibodies**, Monoclonal--Diagnostic Use--DU; Benzodiazepines --Immunology--IM; Binding Sites; Cattle; GABA--Pharmacology--PD; Neural Inhibition...

Chemical Name: **Antibodies**, Monoclonal; (Benzodiazepines; (Neurotransmitters; (Receptors, GABA-A; (GABA

6/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06077311 85270562

Demonstration of benzodiazepine-like molecules in the mammalian brain with a monoclonal **antibody** to benzodiazepines.

Sangameswaran L; de Blas AL

Proc Natl Acad Sci U S A (UNITED STATES) Aug 1985, 82 (16) p5560-4, ISSN 0027-8424 Journal Code: PV3
Contract/Grant No.: NS 17708, NS, NINDS
Languages: ENGLISH
Document type: JOURNAL ARTICLE

An anti-benzodiazepine monoclonal **antibody** has been used to demonstrate the existence of benzodiazepine-like molecules in the brain. Immunocytochemical experiments show that these molecules are neuronal and not glial and that they are ubiquitously distributed throughout the brain. Immunoblots indicate the presence of benzodiazepine-like epitopes in several brain peptides. Small benzodiazepine-like molecules were isolated from the brain soluble fraction by immunoaffinity chromatography. They block the binding of agonists, inverse agonists, and **antagonists** to the neuronal-type benzodiazepine receptor. The neurotransmitter gamma-aminobutyric acid increases the affinity of the benzodiazepine receptor for the purified endogenous molecules. The results indicate that the immunoaffinity-purified molecules behave like the neuronal-type benzodiazepine receptor agonists. The purified molecules, however, do not inhibit the binding of tritiated Ro 5-4864 to the **"peripheral-type" benzodiazepine receptor** . The results demonstrate the existence of **benzodiazepine**-like molecules in the brain that bind to the benzodiazepine **receptor**. These molecules are different from the endogenous benzodiazepine receptor ligands reported by others.

Demonstration of benzodiazepine-like molecules in the mammalian brain with a monoclonal **antibody** to benzodiazepines.

An anti-benzodiazepine monoclonal **antibody** has been used to demonstrate the existence of benzodiazepine-like molecules in the brain. Immunocytochemical...

... brain soluble fraction by immunoaffinity chromatography. They block the binding of agonists, inverse agonists, and **antagonists** to the neuronal-type benzodiazepine receptor. The neurotransmitter

gamma-aminobutyric acid increases the affinity of...

... purified molecules, however, do not inhibit the binding of tritiated Ro 5-4864 to the "**peripheral-type**" **benzodiazepine receptor**. The results demonstrate the existence of **benzodiazepine** -like molecules in the brain that bind to the benzodiazepine **receptor** . These molecules are different from the endogenous benzodiazepine receptor ligands reported by others.

Descriptors: **Antibodies**, Monoclonal; *Benzodiazepines--Analysis--AN ; *Brain--Cytology--CY; *Receptors, GABA-A--Analysis--AN; Antigen-**Antibody** Complex; Brain--Metabolism--ME; Brain Chemistry; Chromatography, Affinity; Hybridomas--Immunology--IM; Ligands; Mice; Mice, Inbred...

Chemical Name: **Antibodies**, Monoclonal; (Antigen-**Antibody** Complex; (Benzodiazepines; (Ligands

6/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05859455 87063241

Demonstration and purification of an endogenous benzodiazepine from the mammalian brain with a monoclonal **antibody** to benzodiazepines.

De Blas AL; Sangameswaran L

Life Sci (ENGLAND) Nov 24 1986, 39 (21) p1927-36, ISSN 0024-3205 Journal Code: L62
Contract/Grant No.: NS17708, NS, NINDS
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Four hybridoma lines secreting monoclonal **antibodies** to benzodiazepines were produced after BALB/c mice were immunized with a benzodiazepine-bovine serum albumin conjugate. The monoclonal **antibodies** were purified from ascites fluids, and their binding affinities for benzodiazepines and other benzodiazepine receptor ligands were determined. These **antibodies** have very high binding affinities for diazepam, flunitrazepam, Ro5-4864, Ro5-3453, Ro11-6896, and Ro5-3438 (the Kd values are in the 10(-9) M range). However, these **antibodies** have very low affinities for the benzodiazepine receptor inverse agonists (beta-carbolines) and **antagonists** (Ro15-1788 and CGS-8216). One of the monoclonal **antibodies** (21-7F9) has been used to demonstrate the existence of benzodiazepine-like molecules in the brain and for the purification of these molecules. Immunocytochemical experiments show that these molecules are neuronal and not glial and that they are ubiquitously distributed throughout the brain. Immunoblots indicate the presence of benzodiazepine-like epitopes in several brain peptides. An endogenous substance that binds to the central-type benzodiazepine receptor with agonist properties has been purified to homogeneity from the bovine brain. The purification consisted on immunoaffinity chromatography on immobilized monoclonal anti-benzodiazepine **antibody** followed by gel filtration on Sephadex G-25 and two reverse phase HPLCs. The purified substance has a small molecular weight and its activity is protease resistant. The endogenous substance blocks the binding of agonists, inverse agonists and **antagonists** to the central-type benzodiazepine receptor but it does not inhibit the binding of Ro5-4864 to the **peripheral-type benzodiazepine receptor** . The neurotransmitter gamma-aminobutyric acid increases the affinity of the benzodiazepine receptor for the purified substance. Preliminary evidence indicates that the purified substance is a benzodiazepine with a molecular structure that is identical or very close to N-desmethyldiazepam.

Demonstration and purification of an endogenous benzodiazepine from the mammalian brain with a monoclonal **antibody** to benzodiazepines.
Four hybridoma lines secreting monoclonal **antibodies** to benzodiazepines were produced after BALB/c mice were immunized with a

benzodiazepine-bovine serum albumin conjugate. The monoclonal **antibodies** were purified from ascites fluids, and their binding affinities for benzodiazepines and other benzodiazepine receptor ligands were determined. These **antibodies** have very high binding affinities for diazepam, flunitrazepam, Ro5-4864, Ro5-3453, Ro11-6896, and Ro5-3438 (the Kd values are in the 10(-9) M range). However, these **antibodies** have very low affinities for the benzodiazepine receptor inverse agonists (beta-carbolines) and **antagonists** (Ro15-1788 and CGS-8216). One of the monoclonal **antibodies** (21-7F9) has been used to demonstrate the existence of benzodiazepine-like molecules in the...

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... activity is protease resistant. The endogenous substance blocks the binding of agonists, inverse agonists and **antagonists** to the central-type benzodiazepine receptor but it does not inhibit the binding of Ro5-4864 to the **peripheral-type benzodiazepine receptor** . The neurotransmitter gamma-aminobutyric acid increases the affinity of the benzodiazepine receptor for the purified...
; **Antibodies**, Monoclonal--Immunology--IM; **Antibody** Affinity; Benzodiazepines--Immunology--IM; Benzodiazepines--Metabolism--ME; Cattle; Chromatography, Affinity; Chromatography, Gel; Chromatography, High Pressure...
Chemical Name: **Antibodies**, Monoclonal; (Benzodiazepines; (Epitopes; (Receptors, GABA-A; (benzodiazepine

6/3,K,AB/6 (Item 1 from file: 434)
DIALOG(R)File 434:SciSearch(R) Cited Ref Sci

06177591 Genuine Article#: TX675 Number of References: 17
Title: ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL EVIDENCE THAT **PERIPHERAL TYPE BENZODIAZEPINE RECEPTORS** ARE COUPLED TO CALCIUM CHANNELS IN THE HEART
Author(s): MESTRE M; CARRIOT T; BELIN C; UZAN A; RENAULT C; DUBROEUCQ MC; GUEREMY C; DOBLE A; LEFUR G
Corporate Source: GRP RHONE POULENC SANTE,PHARMUKA LABS,35 QUAI MOULIN CAGE/F-92231 GENNEVILLIERS//FRANCE/
Journal: LIFE SCIENCES, 1985, V36, N4, P391-400
Language: ENGLISH Document Type: ARTICLE

Title: ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL EVIDENCE THAT **PERIPHERAL TYPE BENZODIAZEPINE RECEPTORS** ARE COUPLED TO CALCIUM CHANNELS IN THE HEART
...Research Fronts: CHANNELS)
85-1426 001 (STRUCTURE AND BINDING SITES OF ACETYLCHOLINE RECEPTORS AND EFFECTS OF MONOCLONAL-**ANTIBODY**-BINDING ON RECEPTOR FUNCTION IN EXPERIMENTAL MYASTHENIA GRAVIS)
85-2902 001 (BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF BENZODIAZEPINE RECEPTOR **ANTAGONISTS** AND AGONISTS)
85-6570 001 (STUDIES ON MEMBRANE CURRENTS ACROSS POTASSIUM AND SODIUM CHANNELS IN...

```
ds

Set     Items   Description
S1        574   PERIPHERAL(W)TYPE(5N)BENZODIAZEPINE(5N)RECEPTOR? ?
S2     145689   ANTAGONIST
S3        105   S1 AND S2
S4     437722   ANTAGONIST? ?
S5        137   S1 AND S4
S6        134   S5 AND PY<=1998
S7         94   RD (unique items)
? s cancer

      S8  539205  CANCER
? s cancer? or neoplas? or tumor? or malignan?

          567172  CANCER?
         1136251  NEOPLAS?
          825541  TUMOR?
          265684  MALIGNAN?
      S9 1808306  CANCER? OR NEOPLAS? OR TUMOR? OR MALIGNAN?
? s s7 and s9

              94  S7
         1808306  S9
     S10       5  S7 AND S9
? t s10/3,k,ab/1-5


 10/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

09450641   98157936
      Effects of peripheral-type benzodiazepine
receptor antisense knockout on MA-10 Leydig cell proliferation and
steroidogenesis.
  Kelly-Hershkovitz E; Weizman R; Spanier I; Leschiner S; Lahav M;
Weisinger G; Gavish M
  Department of Pharmacology, The Bruce Rappaport Faculty of Medicine,
Technion-Israel Institute of Technology, 31096 Haifa, Israel.
  J Biol Chem (UNITED STATES)  Mar 6 1998,  273 (10) p5478-83,
ISSN 0021-9258  Journal Code: HIV
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  The peripheral-type benzodiazepine receptor (PBR)
is not only widely expressed throughout the body, but it is also
genetically conserved from bacteria to humans. Many functions have been
attributed to it, but its primary role remains a puzzle. In the current
study, we stably transfected cultures of MA-10 Leydig cells with either
control or 18-kDa PBR antisense knockout plasmids. The antisense knockout
vector was driven by the human enkephalin promoter, which contains two cAMP
response elements, such that cAMP treatment of transfected cells could
superinduce 18-kDa PBR antisense RNA transcription and, hence,
down-regulate endogenous 18-kDa PBR mRNA levels. Control and knockout MA-10
cell lines were then compared at the level of receptor binding, thymidine
incorporation, and steroid biosynthesis. Eighteen-kilodalton PBR knockout
reduced the maximal binding capacity of tritium-labeled PBR ligands, and
the affinity of receptors to the ligands remained unaltered. Additionally,
```

24-h accumulation of progesterone was lower in the knockout cells. Exposure of the two cell types to 8-bromo-cAMP resulted in a robust increase in steroid production. However, a complex pattern of steroid accumulation was observed, in which further progestin metabolism was indicated. The later decline in accumulated progesterone as well as the synthesis of androstenedione were different in the two cell types. At the level of cell proliferation, reduction of 18-kDa PBR mRNA showed no effect. Thus, we conclude that the 18-kDa PBR may have a more important role in steroidogenesis than in proliferation in this Leydig cell line.

Effects of peripheral-type benzodiazepine receptor antisense knockout on MA-10 Leydig cell proliferation and steroidogenesis.

Mar 6 1998,

The peripheral-type benzodiazepine receptor (PBR) is not only widely expressed throughout the body, but it is also genetically conserved...

Descriptors: DNA, Antisense--Pharmacology--PD; *Leydig Cells--Metabolism --ME; *Receptors, GABA-A--Antagonists and Inhibitors--AI...; ME; Protein Binding; Receptors, GABA-A--Physiology--PH; RNA, Messenger --Metabolism--ME; Transfection--Genetics--GE; Tumor Cells, Cultured

10/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09099788 97228770

Hemin-induced erythroid differentiation of human myeloleukemia K562 cell line and its modification by bioresponse modifiers.

Nakajima O; Iwasaki S; Hashimoto Y

Institute of Molecular and Cellular Biosciences, University of Tokyo, Japan.

Cell Mol Biol (Noisy-le-grand) (FRANCE) Feb 1997, 43 (1) p115-34
, Journal Code: BNA

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have found that protoporphyrin IX, which had been regarded as inactive, induces erythroid differentiation. The differentiation-inducing activities of various hemin-related compounds, including hematoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX and protoporphyrin IX dimethyl ester, suggested certain structural requirements for the activity: 1) the iron moiety of hemin is not essential, and 2) the propionic acid side chains of hemin play an important role. In addition, we have examined the influence of some bioactive factors on hemin/protoporphyrin IX-induced differentiation of K562 cell line. Retinoids and tubulin-disruptors dose-dependently enhanced hemin/protoporphyrin IX-induced differentiation of K562 cells, though they did not themselves induce differentiation. Retinoid antagonists suppressed hemin-induced differentiation. The effects of hemin and/or retinoids on the mRNA expressions of oncogenes (c-myc and c-myb) and retinoic acid receptor genes (rar alpha and rar beta) of K562 cells were analyzed. We also examined the possible involvement of peripheral-type benzodiazepine receptor (PBR) in hemin/protoporphyrin IX-induced differentiation of K562 cells by the use of its ligands. Diazepam itself was revealed to possess differentiation-inducing activity on K562 cells. The PBR-specific ligands modified hemin-induced differentiation. These results suggest a requirement for retinoids (or retinoid-like cofactors) for hemin/protoporphyrin IX-induced differentiation of K562 cells and the involvement of PBR in erythroid differentiation of K562 cell line.

Feb 1997,

... protoporphyrin IX-induced differentiation of K562 cells, though they did not themselves induce differentiation. Retinoid antagonists suppressed hemin-induced differentiation. The effects of hemin and/or

retinoids on the mRNA expressions...

...and rar beta) of K562 cells were analyzed. We also examined the possible involvement of peripheral-type benzodiazepine receptor (PBR) in hemin/protoporphyrin IX-induced differentiation of K562 cells by the use of its...
...; myc--Genetics--GE; Retinoids--Pharmacology--PD; RNA, Messenger; Trans-Activators--Genetics--GE; Tubulin--Metabolism--ME; Tumor Cells, Cultured

10/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06937514 91139659
Hormone-stimulated steroidogenesis is coupled to mitochondrial benzodiazepine receptors. Tropic hormone action on steroid biosynthesis is inhibited by flunitrazepam.
Papadopoulos V; Nowzari FB; Krueger KE
Department of Anatomy and Cell Biology, Georgetown University School of Medicine, Washington, D.C. 20007.
J Biol Chem (UNITED STATES) Feb 25 1991, 266 (6) p3682-7, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: RR-05360, RR, NCRR; MH44284, MH, NIMH
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The mitochondrial (peripheral-type) benzodiazepine receptor (MBR) is a drug binding site associated with outer mitochondrial membranes which is coupled to intramitochondrial cholesterol transport, the rate-determining step of steroid biosynthesis. To examine the relationship between MBR function and steroid synthesis regulated by polypeptide hormones, the Y-1 adrenocortical and MA-10 Leydig cell lines were used as model systems responsive to adrenocorticotropin and human choriogonadotropin, respectively. Flunitrazepam, a benzodiazepine which binds to MBR with high nanomolar affinity, inhibited the steroidogenic activity of these hormones, or the activation by 1 mM dibutyryl cAMP, in both cell lines by 30-60% with an IC50 of 500-1000 nM. Scatchard analysis in both cell lines revealed one class of specific binding sites for [3H] flunitrazepam verified as being MBR by displacement studies with a series of MBR ligands. The potencies of these ligands to compete against the antagonism of hormone-stimulated steroidogenesis by flunitrazepam correlated significantly with their abilities to compete against [3H]flunitrazepam binding to MBR (r = 0.99). An inhibition in pregnenolone formation was also observed in isolated mitochondrial preparations characterized as a reduction of cholesterol transport to inner mitochondrial membranes. These observations provide unequivocal evidence that the antagonistic action of flunitrazepam is mediated through its interaction with MBR demonstrating that these drug recognition sites are coupled to steroid biosynthesis activated by tropic hormones.

Feb 25 1991,
The mitochondrial (peripheral-type) benzodiazepine receptor (MBR) is a drug binding site associated with outer mitochondrial membranes which is coupled to...
; Binding, Competitive; Biological Transport; Cell Membrane--Metabolism --ME; Cholesterol--Metabolism--ME; Mice; Pregnenolone--Antagonists and Inhibitors--AI; Pregnenolone--Biosynthesis--BI; Steroids--Antagonists and Inhibitors--AI; Tumor Cells, Cultured

10/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05818639 90127028
Peripheral benzodiazepine binding sites in Nb 2 node lymphoma cells: effects on prolactin-stimulated proliferation and ornithine decarboxylase activity.
Laird HE 2d; Gerrish KE; Duerson KC; Putnam CW; Russell DH
Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, 85721.
Eur J Pharmacol (NETHERLANDS) Nov 14 1989, 171 (1) p25-35, ISSN 0014-2999 Journal Code: EN6
Contract/Grant No.: YG-9290; ES-03587, ES, NIEHS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
3H]Ro 5-4864 binds to Nb 2 node lymphoma cells in a specific saturable and reversible fashion. Scatchard analysis of specific binding data reveals a single, homogeneous class of whole cell binding sites with a Kd of 3.94 +/- 0.22 nM and a Bmax value of 155 +/- 11 fmol (Ro 5-4864 bound)/2 x 10(6) cells. Ro 5-4864, a reported peripheral benzodiazepine receptor agonist both inhibits (10(-6) M) and potentiates (10(-9) M) the mitogenic action of prolactin on the Nb 2 node lymphoma cells. Interestingly, PK 11195, an antagonist , potentiates (10(-9) M) the mitogenic activity of prolactin in these cells. The actions of both Ro 5-4864 and PK 11195 seem to be mediated through a common receptor type since a 10(-6) M concentration of either agent will block the others potentiating action. Furthermore, the simultaneous addition of a 10(-9) M concentration of Ro 5-4864 and PK 11195 does not further increase the effect on prolactin stimulated mitogenesis. Clonazepam, a central benzodiazepine receptor agonist has no effect on prolactin-stimulated mitogenesis in this system. These data suggest that the Nb 2 node lymphoma cells possess a peripheral-type benzodiazepine receptor. In these cells, this receptor seems to serve the function of modulating the ability of the growth factor, prolactin to initiate the mitogenic process. These studies also suggest that Ro 5-4864 is functioning as a partial agonist rather than a 'pure' agonist for the peripheral benzodiazepine receptor in this system.

Nov 14 1989,
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...; Kinetics; Lymph Nodes--Cytology--CY; Lymph Nodes--Enzymology--EN; Lymphoma--Enzymology--EN; Thymidine--Metabolism--ME; Tumor Cells, Cultured--Drug Effects--DE; Tumor Cells, Cultured--Metabolism--ME

10/3,K,AB/5 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Preiviews(R)

08783001 BIOSIS NO.: 199395072352
Diazepam binding inhibitor is a paracrine/autocrine regulator of Leydig cell proliferation and steroidogenesis: Action via peripheral-type benzodiazepine receptor and independent mechanisms.

AUTHOR: Garnier Martine; Boujrad Noureddine; Oke Bankole O; Brown A Shane; Riond Joelle; Ferrara Pascual; Shoyab Mohamed; Suarez-Quian Carlos A; Papadopoulos Vassilios(a)
AUTHOR ADDRESS: (a)Dep. Anatomy Cell Biol., Georgetown Univ. Med. Center, 3900 Reservoir Rd. NW, Washington, D.C. 2, USA

JOURNAL: Endocrinology 132 (1):p444-458 1993
ISSN: 0013-7227
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Previous studies demonstrated that the polypeptide diazepam binding inhibitor (DBI) and its receptor, the peripheral-type benzodiazepine receptor (PBR), are involved in the regulation of steroid biosynthesis and that one site of PBR action resides in mitochondria. In the present investigation, evidence is presented that a functional form of PBR is also present at the cell surface. First, PBR was immunolocalized in the rat testis using biotin-streptavidin peroxidase immunocytochemistry, and results revealed that PBR was present exclusively in the interstitial Leydig cells. Next, the distribution of PBR in MA-10 Leydig cells was further examined using confocal microscopy. MA-10 cells were either fixed and immunostained or fixed/permeabilized and immunostained for PRB followed by generation of confocal microscope optical sections, three-dimensional reconstructions of these sections, and then generation of vertical confocal sections of the three-dimensional reconstruction. In the fixed/unpermeabilized cells, PBR immunostaining at the cell surface was clearly evident, whereas in the fixed/permeabilized cells, intracellular PBR distribution was more robust. These results suggest that the plasma membrane fraction of the receptor could mediate the action of extracellular PBR ligands on Leydig cell function. Next, we examined whether DBI, the naturally occurring PBR ligand, is secreted by testicular cells and whether it could activate the cell surface PBR. Immunolocalization of DBI demonstrated that it was present in both Leydig and Sertoli cells. Further, using an immunoblot assay, we demonstrated that DBI is present in rat testicular interstitial fluid. Metabolic labeling of cultured immature rat Sertoli cells and MA-10 mouse tumor Leydig cells, followed by immunoprecipitation of the secreted proteins with an anti-DBI antiserum, demonstrated that both Leydig and Sertoli cells secrete DBI and could serve as a cell source for the interstitial fluid DBI. Then, we partially purified the DBI present in conditioned medium and interstitial fluid by reverse phase chromatography and demonstrated it to be bioactive, based on displacement of a radiolabeled benzodiazepine (Ro5-4864)-specific ligand for PBR, pronase treatment of different preparations eliminated all bioactivity. We then examined the effects of DBI on Leydig cell function. DBI added to MA-10 cells affected DNA synthesis and cell growth in a biphasic manner; at low concentrations (1 nM), DBI was mitogenic, increasing (3H)thymidine incorporation and cell numbers by 30-40%, while at high concentrations (1 mu-M), DBI inhibited cell growth (30-40%). Similar effects on cell growth were obtained using the benzodiazepine Ro5-4864. The effects of both DBI and Ro5-4864 were inhibited by the antagonist isoquinoline carboxamide PK 11195, suggesting that their actions on cell proliferation were mediated through PBR. DBI directly added to MA-10 or to purified rat Leydig cells also stimulated basal steroid production and potentiated submaxinally hCG-stimulated steroidogenesis (by 2- to 3-fold) in a dose-dependent manner, with an EC-50 of 10 nM. However, the steroidogenic action of DBI was not blocked by PK 11195, but was mimicked by the octadecaneuropeptide (DBI-(33-50)), a peptide with very low affinity for PBR. Because of the widespread occurance of both DBI and PBR in different tissues, we investigated whether DBI may also regulate cell growth and steroid synthesis in other cell models that contain PBR, such as Swiss 3T3 fibroblasts and bovine adrenocortical cells, respectively. The data obtained clearly indicate that activation of PBR by DBI also alters 3T3 fibroblasts cell proliferation, but not effect of DBI on steroid production in adrenocortical cells was observed. These results demonstrate that 1) DBI is secreted by both Leydig and Sertoli cells and is present in the testicular interstitial fluid; 2) DBI, presumably acting via plasma membrane PBR, affects Leydig cell and 3T3 fibroblast DNA synthesis and growth; and 3) DBI, acting via PBR-mediated and/or -independent mechanisms, stimulates Leydig cell steroid production. Thus,

we propose that DBI acts as an autocrine/apracrine regulator of Leydig cell function.

...binding inhibitor is a paracrine/autocrine regulator of Leydig cell proliferation and steroidogenesis: Action via peripheral-type benzodiazepine receptor and independent mechanisms.

ABSTRACT: Previous studies demonstrated that the polypeptide diazepam binding inhibitor (DBI) and its receptor, the peripheral-type benzodiazepine receptor (PBR), are involved in the regulation of steroid biosynthesis and that one site of PBR...

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...benzodiazepine Ro5-4864. The effects of both DBI and Ro5-4864 were inhibited by the antagonist isoquinoline carboxamide PK 11195, suggesting that their actions on cell proliferation were mediated through PBR...

1993

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Set     Items   Description
S1        574   PERIPHERAL(W)TYPE(5N)BENZODIAZEPINE(5N)RECEPTOR? ?
S2     145689   ANTAGONIST
S3        105   S1 AND S2
S4     437722   ANTAGONIST? ?
S5        137   S1 AND S4
S6        134   S5 AND PY<=1998
S7         94   RD (unique items)
S8     539205   CANCER
S9    1808306   CANCER? OR NEOPLAS? OR TUMOR? OR MALIGNAN?
S10         5   S7 AND S9
? s proliferat?

     S11  244545  PROLIFERAT?
? s s7 and s11

              94  S7
          244545  S11
     S12       6  S7 AND S11
? t s12/3,k,ab/1-6


 12/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

09450641   98157936
      Effects     of    peripheral-type    benzodiazepine
receptor  antisense knockout on MA-10 Leydig cell proliferation
 and steroidogenesis.
  Kelly-Hershkovitz  E;  Weizman  R;  Spanier  I;  Leschiner  S;  Lahav  M;
Weisinger G; Gavish M
  Department  of  Pharmacology,  The  Bruce  Rappaport Faculty of Medicine,
Technion-Israel Institute of Technology, 31096 Haifa, Israel.
  J Biol Chem (UNITED STATES)   Mar 6 1998,  273 (10) p5478-83,
ISSN 0021-9258   Journal Code: HIV
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  The peripheral-type benzodiazepine receptor (PBR)
is  not  only  widely  expressed throughout the body, but it is also
genetically conserved from bacteria to humans. Many functions have been
attributed  to  it,  but  its primary role remains a puzzle. In the current
study,  we  stably  transfected  cultures of MA-10 Leydig cells with either
control  or  18-kDa PBR antisense knockout plasmids. The antisense knockout
vector was driven by the human enkephalin promoter, which contains two cAMP
response  elements,  such  that  cAMP  treatment of transfected cells could
superinduce  18-kDa  PBR  antisense  RNA  transcription  and,  hence,
down-regulate endogenous 18-kDa PBR mRNA levels. Control and knockout MA-10
cell  lines  were then compared at the level of receptor binding, thymidine
incorporation,  and  steroid biosynthesis. Eighteen-kilodalton PBR knockout
reduced  the  maximal  binding capacity of tritium-labeled PBR ligands, and
the  affinity of receptors to the ligands remained unaltered. Additionally,
24-h accumulation of progesterone was lower in the knockout cells. Exposure
of  the  two  cell  types  to 8-bromo-cAMP resulted in a robust increase in
steroid  production. However, a complex pattern of steroid accumulation was
observed,  in  which  further progestin metabolism was indicated. The later
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decline in accumulated progesterone as well as the synthesis of androstenedione were different in the two cell types. At the level of cell proliferation , reduction of 18-kDa PBR mRNA showed no effect. Thus, we conclude that the 18-kDa PBR may have a more important role in steroidogenesis than in proliferation in this Leydig cell line.

Effects of peripheral-type benzodiazepine receptor antisense knockout on MA-10 Leydig cell proliferation and steroidogenesis.
Mar 6 1998,
The peripheral-type benzodiazepine receptor (PBR) is not only widely expressed throughout the body, but it is also genetically conserved...
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Descriptors: DNA, Antisense--Pharmacology--PD; *Leydig Cells--Metabolism --ME; *Receptors, GABA-A--Antagonists and Inhibitors--AI

12/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07730894 94132488
In vitro inhibition of cellular immune responses by benzodiazepines and PK 11195. Effects on mitogen- and alloantigen-driven lymphocyte proliferation and on IL-1, IL-2 synthesis and IL-2 receptor expression.
Ramseier H; Lichtensteiger W; Schlumpf M
Institute for Immunology and Virology, University of Zurich, Switzerland.
Immunopharmacol Immunotoxicol (UNITED STATES) Nov 1993, 15 (5) p557-82, ISSN 0892-3973 Journal Code: IAI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In vitro mitogen-driven lymphocyte proliferation tests (Con A, LPS) on murine lymph node and spleen cells revealed inhibition of T and B cell stimulation by different benzodiazepines and by PK 11195, with IC50 values in the low micromolar range. T cell responses as a consequence of recognition of alloantigens, as measured in mixed lymphocyte cultures (MLC), were affected in an analogous way. In all systems, agonists at peripheral type benzodiazepine receptors (Ro 5-4864 and the non-benzodiazepine compound PK 11195) and diazepam which acts on both, central and peripheral type benzodiazepine receptors , were most potent; clonazepam, a central type agonist, proved about half as active. The central type antagonist Ro 15-1788 failed to antagonize the action of diazepam and clonazepam. Variations among cells from several congenic strains of mice were modest. Cytotoxicity could not be made responsible for drug effects. The most susceptible stage of mitogen-triggered T and B lymphocyte proliferation was found to be at incipience. Radioresistant, adherent spleen cells, upon LPS-stimulation formed only small amounts of the cytokine IL-1. Its release was affected only at very high drug concentrations. Similar small amounts of IL-1 were generated during MLC; in this case, the drugs were about 10 times less potent than in mitogen-induced proliferation assays. Peripheral agonists were more active on IL-1 synthesis. Spleen cells stimulated with Con A and cultivated with the highest concentration of diazepam and clonazepam formed markedly greater amounts of IL-2 than those cultivated in medium, while at this concentration PK 11195 allowed no formation of the lymphokine.(ABSTRACT TRUNCATED AT 250 WORDS)

...cellular immune responses by benzodiazepines and PK 11195. Effects on mitogen- and alloantigen-driven lymphocyte proliferation and on IL-1,

IL-2 synthesis and IL-2 receptor expression.
Nov 1993,
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12/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06974419 92136119
Peripheral-type benzodiazepines inhibit proliferation of astrocytes in culture.
Bruce JH; Ramirez AM; Lin L; Oracion A; Agarwal RP; Norenberg MD
Department of Pathology, University of Miami, FL.
Brain Res (NETHERLANDS) Nov 8 1991, 564 (1) p167-70, ISSN 0006-8993 Journal Code: B5L
Contract/Grant No.: NS 24853, NS, NINDS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Peripheral-type benzodiazepine (BZD) receptors have been identified in brain and are predominantly localized to astrocytes. To determine their potential role in controlling astroglial proliferation , DNA synthesis, growth curves and mitotic index were investigated in primary astrocyte cultures which had been exposed to Ro5-4864 (a peripheral-type BZD ligand) and PK11195 (a peripheral-type BZD receptor antagonist). There was a dose-dependent inhibition of mitosis when two-week-old cells in culture were exposed to 50 nM, 500 nM, 1 microM and 10 microM Ro5-4864 for 24 h. Exposure of 5-, 8-, 12- and 15-day-old cultures to Ro5-4864 and PK11195 for 24 h did not affect growth rate and DNA synthesis; however, continuous exposure to 10 microM Ro5-4864 caused a persistent inhibition of cell growth and [3H]thymidine incorporation (P less than 0.05) while nanomolar concentrations did not cause any significant change. Concurrent administration of Ro5-4864 with PK11195 resulted in a partial reversal of Ro5-4864-induced inhibition in DNA synthesis and mitosis. These results indicate that peripheral-type BZDs are capable of inhibiting proliferation of astrocytes in culture.

Peripheral-type benzodiazepines inhibit proliferation of astrocytes in culture.
Nov 8 1991,
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12/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06145866 89116973
Central-type and peripheral-type benzodiazepine receptors.
Saano V
Department of Pharmacology and Toxicology, University of Kuopio, Finland.
Ann Clin Res (FINLAND) 1988, 20 (5) p348-55, ISSN 0003-4762
Journal Code: 53A
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The benzodiazepines had already been in wide use as anxiolytics and anticonvulsants for more than ten years before their site of action in the central nervous system, the benzodiazepine receptor, was discovered. Simultaneously, a binding site in the peripheral organs, e.g. heart, lungs and kidneys, was found. Although some benzodiazepines, such as diazepam, bind to both central and peripheral benzodiazepine receptors with a high affinity, these two binding sites exhibit quite different properties. It is already clear that the central benzodiazepine receptors are in many regions of the brain coupled with the receptors for gamma-amino butyric acid, and they mediate the acute actions of benzodiazepines in the central nervous system. Through them opposite effects, such as anxiety and convulsions, can also be evoked by using inverse agonists, e.g. some beta-carbolines. All these effects can be blocked with benzodiazepine receptor antagonists , some of which are already used as specific antidotes against benzodiazepine overdose. The multitude of pharmacological effects that can be produced through central benzodiazepine receptors provides a good opportunity for the development of new drugs. The role of the peripheral-type receptors is less clear, but it seems that they are connected with more slowly-appearing drug actions, such as modulation of cell proliferation . Now that endogenous ligands for both the central-type (a peptide called diazepam binding inhibitor; DBI) and for the peripheral-type (porphyrins) benzodiazepine receptors%
%% have been discovered, even more productive research can be expected.

Central-type and peripheral-type benzodiazepine receptors.
1988,
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12/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05818639 90127028
Peripheral benzodiazepine binding sites in Nb 2 node lymphoma cells: effects on prolactin-stimulated **proliferation** and ornithine decarboxylase activity.
Laird HE 2d; Gerrish KE; Duerson KC; Putnam CW; Russell DH
Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, 85721.
Eur J Pharmacol (NETHERLANDS) Nov 14 **1989**, 171 (1) p25-35, ISSN 0014-2999 Journal Code: EN6
Contract/Grant No.: YG-9290; ES-03587, ES, NIEHS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
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Peripheral benzodiazepine binding sites in Nb 2 node lymphoma cells: effects on prolactin-stimulated **proliferation** and ornithine decarboxylase activity.
Nov 14 **1989**,
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12/3,K,AB/6 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Preiviews(R)

08783001 BIOSIS NO.: 199395072352
Diazepam binding inhibitor is a paracrine/autocrine regulator of Leydig cell **proliferation** and steroidogenesis: Action via **peripheral -type benzodiazepine receptor** and independent mechanisms.

AUTHOR: Garnier Martine; Boujrad Noureddine; Oke Bankole O; Brown A Shane; Riond Joelle; Ferrara Pascual; Shoyab Mohamed; Suarez-Quian Carlos A; Papadopoulos Vassilios(a)
AUTHOR ADDRESS: (a)Dep. Anatomy Cell Biol., Georgetown Univ. Med. Center, 3900 Reservoir Rd. NW, Washington, D.C. 2, USA

JOURNAL: Endocrinology 132 (1):p444-458 1993

ISSN: 0013-7227
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Previous studies demonstrated that the polypeptide diazepam binding inhibitor (DBI) and its **receptor**, the **peripheral-type benzodiazepine receptor** (PBR), are involved in the regulation of steroid biosynthesis and that one site of PBR action resides in mitochondria. In the present investigation, evidence is presented that a functional form of PBR is also present at the cell surface. First, PBR was immunolocalized in the rat testis using biotin-streptavidin peroxidase immunocytochemistry, and results revealed that PBR was present exclusively in the interstitial Leydig cells. Next, the distribution of PBR in MA-10 Leydig cells was further examined using confocal microscopy. MA-10 cells were either fixed and immunostained or fixed/permeabilized and immunostained for PRB followed by generation of confocal microscope optical sections, three-dimensional reconstructions of these sections, and then generation of vertical confocal sections of the three-dimensional reconstruction. In the fixed/unpermeabilized cells, PBR immunostaining at the cell surface was clearly evident, whereas in the fixed/permeabilized cells, intracellular PBR distribution was more robust. These results suggest that the plasma membrane fraction of the receptor could mediate the action of extracellular PBR ligands on Leydig cell function. Next, we examined whether DBI, the naturally occurring PBR ligand, is secreted by testicular cells and whether it could activate the cell surface PBR. Immunolocalization of DBI demonstrated that it was present in both Leydig and Sertoli cells. Further, using an immunoblot assay, we demonstrated that DBI is present in rat testicular interstitial fluid. Metabolic labeling of cultured immature rat Sertoli cells and MA-10 mouse tumor Leydig cells, followed by immunoprecipitation of the secreted proteins with an anti-DBI antiserum, demonstrated that both Leydig and Sertoli cells secrete DBI and could serve as a cell source for the interstitial fluid DBI. Then, we partially purified the DBI present in conditioned medium and interstitial fluid by reverse phase chromatography and demonstrated it to be bioactive, based on displacement of a radiolabeled benzodiazepine (Ro5-4864)-specific ligand for PBR, pronase treatment of different preparations eliminated all bioactivity. We then examined the effects of DBI on Leydig cell function. DBI added to MA-10 cells affected DNA synthesis and cell growth in a biphasic manner; at low concentrations (1 nM), DBI was mitogenic, increasing (3H)thymidine incorporation and cell numbers by 30-40%, while at high concentrations (1 mu-M), DBI inhibited cell growth (30-40%). Similar effects on cell growth were obtained using the benzodiazepine Ro5-4864. The effects of both DBI and Ro5-4864 were inhibited by the **antagonist** isoquinoline carboxamide PK 11195, suggesting that their actions on cell **proliferation** were mediated through PBR. DBI directly added to MA-10 or to purified rat Leydig cells also stimulated basal steroid production and potentiated submaxinally hCG-stimulated steroidogenesis (by 2- to 3-fold) in a dose-dependent manner, with an EC-50 of 10 nM. However, the steroidogenic action of DBI was not blocked by PK 11195, but was mimicked by the octadecaneuropeptide (DBI-(33-50)), a peptide with very low affinity for PBR. Because of the widespread occurance of both DBI and PBR in different tissues, we investigated whether DBI may also regulate cell growth and steroid synthesis in other cell models that contain PBR, such as Swiss 3T3 fibroblasts and bovine adrenocortical cells, respectively. The data obtained clearly indicate that activation of PBR by DBI also alters 3T3 fibroblasts cell **proliferation**, but not effect of DBI on steroid production in adrenocortical cells was observed. These results demonstrate that 1) DBI is secreted by both Leydig and Sertoli cells and is present in the testicular interstitial fluid; 2) DBI, presumably acting via plasma membrane PBR, affects Leydig cell and 3T3 fibroblast DNA synthesis and growth; and 3) DBI, acting via PBR-mediated and/or -independent mechanisms, stimulates Leydig cell steroid production. Thus, we propose that DBI acts as an

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Diazepam binding inhibitor is a paracrine/autocrine regulator of Leydig cell **proliferation** and steroidogenesis: Action via **peripheral -type benzodiazepine receptor** and independent mechanisms.

ABSTRACT: Previous studies demonstrated that the polypeptide diazepam binding inhibitor (DBI) and its **receptor**, the **peripheral-type benzodiazepine receptor** (PBR), are involved in the regulation of steroid biosynthesis and that one site of PBR...

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     $0.03  Estimated total session cost   0.198 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2000/Apr W4
         (c) format only 2000 Dialog Corporation
  File  55:Biosis Previews(R)  1993-2000/Mar W2
         (c) 2000 BIOSIS
*File  55: Updates renamed. See Help News55.
  File  34:SciSearch(R) Cited Ref Sci  1990-2000/Mar W1
         (c) 2000 Inst for Sci Info
  File 434:SciSearch(R) Cited Ref Sci  1974-1989/Dec
         (c) 1998 Inst for Sci Info
  File 340:CLAIMS(R)/US Patent  1950-00/Feb 29
         (c) 2000 IFI/CLAIMS(r)
*File 340: *** UD=20000111 and 20000118 have some incorrectly
attributed foreign priorities.  See HELP NEWS 340 for details.

      Set  Items  Description
      ---  -----  -----------
? s antisense(%N)oligonucleotide??

      S1       0  ANTISENSE(%N)OLIGONUCLEOTIDE??
? s antisense(5n)oligonucleotide? ?

           39224  ANTISENSE
           96809  OLIGONUCLEOTIDE? ?
      S2   16290  ANTISENSE(5N)OLIGONUCLEOTIDE? ?
? s vector

      S3  197122  VECTOR
? s s2 and s3

           16290  S2
          197122  S3
      S4     511  S2 AND S3
? s express?

      S5 1875942  EXPRESS?
? s s4 and s5

             511  S4
         1875942  S5
      S6     432  S4 AND S5
? s inhibit? or reduc?

Processing
         2011939  INHIBIT?
         2372434  REDUC?
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      S7 3975334  INHIBIT? OR REDUC?
? s s6 and s7

             432  S6
         3975334  S7
      S8     302  S6 AND S7
? s s8 and py<1998

Processing
Processing
             302  S8
        31227784  PY<1998
      S9     204  S8 AND PY<1998
? t s9/3,k,ab/1-10


 9/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09823768   99035164
  Endogenous interleukin 6 can function as an in vivo growth- stimulatory
factor for advanced-stage human melanoma cells.
  Lu C; Sheehan C; Rak JW; Chambers CA; Hozumi N; Kerbel RS
  Division of Cancer Biology Research, Reichmann Research Building,
Sunnybrook Health Science Centre, 2075 Bayview Avenue, Toronto, Ontario M4N
3M5.
  Clin Cancer Res (UNITED STATES) Aug 1996, 2 (8) p1417-25, ISSN
1078-0432 Journal Code: C2H
  Contract/Grant No.: CA-41233, CA, NCI
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  We have previously shown that a majority of human melanoma cell lines
derived from early-stage lesions were growth inhibited by exogenous
interleukin 6 (IL-6) in vitro, whereas cell lines from advanced-stage
lesions were resistant to such IL-6-induced growth inhibition. Among
the resistant melanoma cell lines, 50-60% constitutively produced IL-6,
which appeared to function as a growth stimulator in vitro, based on the
growth-suppressive effects of antisense oligonucleotides to the
IL-6 gene. The present study was primarily aimed at evaluating whether
endogenous IL-6 also functions in vivo as a growth modulator for
IL-6-producing and -nonproducing melanoma cells. To do so, we first
introduced an IL-6 expression vector into IL-6-nonproducing
human melanoma cells using WM35, an early-stage (radial growth phase) cell
line, the growth of which is normally inhibited by IL-6, and WM983A,
an advanced-stage cell line, the growth of which in vitro is not affected
by exogenous IL-6. None of the IL-6-producing transfectants showed a
significant alteration in tumor growth in nude mice. Next, two
IL-6-producing melanoma cell lines, both of which were derived from
metastases, MeWo and WM9, and which are growth resistant to exogenously
added IL-6, were transfected with an antisense IL-6 expression
vector . Several transfectant clones manifested a constitutive
decrease in IL-6 gene expression and protein production, and they
also gave rise to much smaller tumors with slower growth rates and longer
latency periods. However, these IL-6 antisense transfectants were not
growth suppressed in in vitro cell cultures, relative to their respective
parental controls. Taken together, the results demonstrate that endogenous
IL-6 can indeed function as a growth stimulator for human cutaneous
melanomas in vivo. This growth-stimulatory or survival mechanism remains to
be clarified but may be paracrine rather than autocrine in nature.

  Aug 1996,
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lesions were growth inhibited by exogenous interleukin 6 (IL-6) in
vitro, whereas cell lines from advanced-stage lesions were resistant to
```

such IL-6-induced growth **inhibition** . Among the resistant melanoma cell lines, 50-60% constitutively produced IL-6, which appeared to function as a growth stimulator in vitro, based on the growth-suppressive effects of **antisense oligonucleotides** to the IL-6 gene. The present study was primarily aimed at evaluating whether endogenous...

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...are growth resistant to exogenously added IL-6, were transfected with an antisense IL-6 **expression vector**. Several transfectant clones manifested a constitutive decrease in IL-6 gene **expression** and protein production, and they also gave rise to much smaller tumors with slower growth...

; Down-Regulation (Physiology); Interleukin-6--Genetics--GE; Mice; Mice, Nude; Neovascularization, Pathologic; **Oligonucleotides, Antisense**--Pharmacology--PD; Tumor Cells, Cultured

Chemical Name: Growth Substances; (Interleukin-6; (**Oligonucleotides , Antisense**

9/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09432926 98124580

Regulation of cholinesterase gene **expression** affects neuronal differentiation as revealed by transfection studies on reaggregating embryonic chicken retinal cells.

Robitzki A; Mack A; Hoppe U; Chatonnet A; Layer PG

Department of Developmental and Neurobiology, Institute for Zoology, University of Technology, Darmstadt, Germany.

Eur J Neurosci (ENGLAND) Nov **1997**, 9 (11) p2394-405, ISSN 0953-816X Journal Code: BYG

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In the embryonic chicken neuroepithelium, butyrylcholinesterase (BChE) as a proliferation marker and then acetylcholinesterase (AChE) as a differentiation marker are **expressed** in a mutually exclusive manner. These and other data indicate a coregulation of cholinesterase **expression** , and also possible roles of cholinesterases during neurogenesis. Here, both aspects are investigated by two independent transfection protocols of dissociated retina cells of the 6-day-old chick embryo in reaggregation culture, both protocols leading to efficient overexpression of AChE protein. The effect of the overexpressed AChE protein on the re-establishment of retina-like three-dimensional networks (so-called retinospheroids) was studied. In a first approach, we transfected retinospheroids with a pSVK3 **expression vector** into which a cDNA construct encoding the entire rabbit AChE gene had been inserted in sense orientation. As detected at the mRNA level, rabbit AChE was heterologously overexpressed in chicken retinospheroids. Remarkably, this was accompanied by a strong increase in endogenous chicken AChE protein, while the total AChE activity was only slightly increased. This increase was due to chicken enzyme, as shown by species-specific **inhibition** studies using fasciculin. Clearly, total AChE activity is regulated post-translationally. As an alternative method of AChE overexpression, transfection of spheroids was performed with an antisense-5'-BChE **vector** , which not only resulted in the down-regulation of BChE **expression** , but also strongly increased chicken AChE transcripts, protein and enzyme activity. Histologically, a higher concentration of AChE protein (as a consequence of either AChE overexpression or BChE suppression) was associated with an advanced degree

of tissue differentiation, as detected by immunostaining for the cytoskeletal protein vimentin.

Regulation of cholinesterase gene **expression** affects neuronal differentiation as revealed by transfection studies on reaggregating embryonic chicken retinal cells.
Nov **1997**,
... butyrylcholinesterase (BChE) as a proliferation marker and then acetylcholinesterase (AChE) as a differentiation marker are **expressed** in a mutually exclusive manner. These and other data indicate a coregulation of cholinesterase **expression**, and also possible roles of cholinesterases during neurogenesis. Here, both aspects are investigated by two...

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Descriptors: Cholinesterases--Biosynthesis--BI; *Cholinesterases --Genetics--GE; *Gene **Expression** Regulation, Enzymologic--Physiology --PH; *Neurons--Physiology--PH; *Retina--Enzymology--EN...; Embryo; DNA --Analysis--AN; Electrophoresis, Polyacrylamide Gel; Eye Proteins --Biosynthesis--BI; Immunohistochemistry; Neurons--Enzymology--EN; **Oligonucleotides, Antisense**--Diagnostic Use--DU; Rabbits; Retina--Cytology--CY; Retina--Embryology--EM; RNA--Biosynthesis--BI; RNA --Isolation...
Chemical Name: Cholinesterases; (Eye Proteins; (**Oligonucleotides, Antisense**; (RNA; (DNA

9/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09362607 98044837
In vivo production of oligodeoxyribonucleotides of specific sequences: application to antisense DNA.
Inouye M; Mao JR; Shimamoto T; Inouye S
Robert Wood Johnson Medical School, Department of Biochemistry, Piscataway, NJ 08854-5635, USA.
Ciba Found Symp (NETHERLANDS) **1997**, 209 p224-33; discussion 233-4, ISSN 0300-5208 Journal Code: D7X
Contract/Grant No.: GM44012, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
Retrons, bacterial retroelements found in Gram-negative bacteria, are integrated into the bacterial genome **expressing** a reverse transcriptase related to eukaryotic reverse transcriptase. The bacterial reverse transcriptases are responsible for the production of multicopy, single-stranded (ms) DNA consisting of a short single-stranded DNA that is attached to an internal guanosine residue of an RNA molecule by a 2',5'-phosphodiester linkage. Reverse transcriptases use an RNA transcript from the retrons, not only as primer, but also as template for msDNA synthesis. By studying the structural requirement, it was found that for msDNA synthesis an internal region of msDNA can be replaced with other sequences. msDNA can thus be used as a **vector** for in vivo production

of an oligodeoxyribonucleotide of a specific sequence. Artificial msDNAs containing a sequence complementary to part of the mRNA for the major outer membrane lipoprotein of Escherichia coli effectively **inhibited** lipoprotein biosynthesis upon induction of msDNA synthesis. This is the first demonstration of in vivo synthesis of oligodeoxyribonucleotides having antisense function. Since we have previously demonstrated that bacterial retrons are functional in eukaryotes producing msDNA in yeast and in mouse NIH/3T3 fibroblasts, the present system may also be used to produce a specific oligodeoxyribonucleotide inside the cells to regulate eukaryotic gene **expression** artificially. We also describe a method to produce cDNA to a specific cellular mRNA using the retron system.

1997,

Retrons, bacterial retroelements found in Gram-negative bacteria, are integrated into the bacterial genome **expressing** a reverse transcriptase related to eukaryotic reverse transcriptase. The bacterial reverse transcriptases are responsible for...

...of msDNA can be replaced with other sequences. msDNA can thus be used as a **vector** for in vivo production of an oligodeoxyribonucleotide of a specific sequence. Artificial msDNAs containing a...
... to part of the mRNA for the major outer membrane lipoprotein of Escherichia coli effectively **inhibited** lipoprotein biosynthesis upon induction of msDNA synthesis. This is the first demonstration of in vivo...

... also be used to produce a specific oligodeoxyribonucleotide inside the cells to regulate eukaryotic gene **expression** artificially. We also describe a method to produce cDNA to a specific cellular mRNA using...

Descriptors: DNA, **Antisense**--Biosynthesis--BI; ***Oligonucleotides**, **Antisense**--Biosynthesis--BI; Bacterial Outer Membrane Proteins --Genetics--GE; DNA, Single-Stranded--Biosynthesis--BI; Gene **Expression%%%; Mice; Molecular Sequence Data**

Chemical Name: Bacterial Outer Membrane Proteins; (DNA, Antisense; (DNA, Single-Stranded; (Lpp protein, E. coli; (Oligonucleotides, Antisense

9/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09344380 97453284

Expression **of a D2 dopamine receptor antisense RNA in brain** inhibits **D2-mediated behaviors.**

Weiss B; Davidkova G; Zhou LW; Zhang SP; Morabito M

Department of Pharmacology, MCP-Hahnemann School of Medicine, Allegheny University of the Health Sciences, Philadelphia, PA 19129, USA. weissb@allegheny.edu

Neurochem Int (ENGLAND) Oct 1997, 31 (4) p571-80, ISSN 0197-0186 Journal Code: BNU

Contract/Grant No.: MH42148, MH, NIMH

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Drugs currently used to treat disorders of dopamine-mediated behaviors in the central nervous system are non-selective in that they interact not only with more than one isoform of dopamine receptor but also with receptors for other neurotransmitters. A new strategy to inhibit the actions of individual dopamine receptor subtypes is to inhibit the synthesis of the receptors through the use of oligonucleotides antisense **to the transcripts encoding the different receptors. Earlier studies showed that oligodeoxynucleotides antisense to the D1 or D2 dopamine receptor messenger RNAs specifically** inhibited **the biological actions mediated by these individual isoforms of the dopamine receptor. However, these actions were relatively short-lasting. To determine whether one can achieve long-lasting** inhibition **of dopamine responses, while still taking**

advantage of the highly selective nature of an antisense strategy, an expression vector was employed that generates antisense RNA to the transcript encoding the D2 dopamine receptor. A single intrastriatal injection of this vector generated an antisense RNA to the D2 dopamine receptor, selectively reduced the levels of D2 dopamine receptors, and caused selective, long-term inhibition of behaviors mediated by D2 dopamine agonists. Such an antisense RNA strategy may find use in studying the function of dopaminergic receptors and in disorders associated with dopaminergic hyperactivity.

Expression of a D2 dopamine receptor antisense RNA in brain inhibits D2-mediated behaviors.
Oct 1997,
... isoform of dopamine receptor but also with receptors for other neurotransmitters. A new strategy to inhibit the actions of individual dopamine receptor subtypes is to inhibit the synthesis of the receptors through the use of oligonucleotides antisense to the transcripts encoding the different receptors. Earlier studies showed that oligodeoxynucleotides antisense to the D1 or D2 dopamine receptor messenger RNAs specifically inhibited the biological actions mediated by these individual isoforms of the dopamine receptor. However, these actions were relatively short-lasting. To determine whether one can achieve long-lasting inhibition of dopamine responses, while still taking advantage of the highly selective nature of an antisense strategy, an expression vector was employed that generates antisense RNA to the transcript encoding the D2 dopamine receptor. A single intrastriatal injection of this vector generated an antisense RNA to the D2 dopamine receptor, selectively reduced the levels of D2 dopamine receptors, and caused selective, long-term inhibition of behaviors mediated by D2 dopamine agonists. Such an antisense RNA strategy may find use...
...; Physiology--PH; Injections; Mice; Mice, Inbred Strains; Oxidopamine --Pharmacology--PD; Receptors, Dopamine D2--Antagonists and Inhibitors--AI; Rotation; Stereotyped Behavior--Drug Effects--DE; Stereotyped Behavior--Physiology--PH

9/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09293575 97474434
Down-regulation of the nm23.h1 gene inhibits cell proliferation.
Cipollini G; Berti A; Fiore L; Rainaldi G; Basolo F; Merlo G; Bevilacqua G; Caligo MA
Department of Oncology, University of Pisa, Italy.
Int J Cancer (UNITED STATES) Oct 9 1997, 73 (2) p297-302, ISSN 0020-7136 Journal Code: GQU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
nm23 gene expression is strictly related to the state of cell growth. The level of its expression parallels the fraction of thymidine-incorporating cells (S-phase cells) in neoplastic mammary tissues and in the synchronously cycling fraction of MCF 10A cells. nm23.h1 reaches a peak of expression in the S-phase, and is present at very low level during the GO/G1 phase. Two strategies are used to demonstrate the direct involvement of the nm23.h1 gene in the process of cell proliferation. The first consists of transient inhibition of nm23.h1 expression by using anti-sense oligonucleotide treatment; weak inhibitory effect on cell proliferation is observed. The second strategy involves the stable inhibition of nm23.h1 expression by transfection of MCF10A cells with a plasmid vector expressing the human nm23.h1 anti-sense mRNA. The anti-sense-transfected cells show consistently slower proliferative activity than the control.

Down-regulation of the nm23.h1 gene inhibits cell proliferation.
Oct 9 1997,

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Descriptors: Breast--Metabolism--ME; *Cell Division--Physiology--PH; *Down-Regulation (Physiology); *Gene Expression Regulation, Enzymologic; *Nucleoside-Diphosphate Kinase--Metabolism--ME; *Transcription Factors--Metabolism--ME...; Drug Effects--DE; Cell Line, Transformed; DNA --Biosynthesis--BI; DNA--Drug Effects--DE; Epithelium; Gene Expression Regulation, Enzymologic--Physiology--PH; Nucleoside-Diphos phate Kinase--Genetics--GE; Oligonucleotides, Antisense --Pharmacology--PD; RNA, Messenger--Drug Effects--DE; Transcription Factors --Genetics--GE; Transfection

Chemical Name: Nucleoside-Diphosphate Kinase; (Nm23 protein; (Oligonucleotides, Antisense; (RNA, Messenger; (Transcription Factors; (DNA

9/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09268944 97313471

Heme oxygenase-mediated resistance to oxygen toxicity in hamster fibroblasts.

Dennery PA; Sridhar KJ; Lee CS; Wong HE; Shokoohi V; Rodgers PA; Spitz DR
Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305, USA. mn.phd@forsythe.stanford.edu

J Biol Chem (UNITED STATES) Jun 6 1997, 272 (23) p14937-42,
ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: HL52701, HL, NHLBI; HL51469, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The role of heme oxygenase (HO)-1 was evaluated in the oxygen-resistant hamster fibroblast cell line, O2R95, which moderately overexpress HO when compared with the parental cell line, HA-1. To suppress HO-1 expression, O2R95 were transfected with HO-1 antisense oligonucleotide or treated with tin-mesoporphyrin (SnMP). To increase HO-1 expression , cells were transfected with HO-1 cDNA in a pRC/cytomegalovirus (CMV) vector . All cells were challenged with a 48-h exposure to 95% O2 (hyperoxia). When HO activity was suppressed, O2R95 cells had significantly decreased cell viability, increased susceptibility to lipid peroxidation, and increased protein oxidation in hyperoxia. In contrast, further overexpression of HO-1 did not improve resistance to oxygen toxicity. Antisense-transfected cells and SnMP-treated cells with lowered HO activity showed increased levels of cellular heme compared with controls. In the HO-1 cDNA-transfected O2R95 cells, cellular heme was lowered compared with controls; however, cellular redox active iron levels were increased. We conclude that HO mediates cytoprotection to oxygen toxicity within a narrow range of expression. We speculate that this protective effect may be mediated in part through increased metabolism of the pro-oxidant heme but that higher levels of HO activity obviate

protection by increased redox active iron release.

Jun 6 1997,

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Descriptors: Antioxidants--Pharmacology--PD; *Heme Oxygenase (Decyclizing)--Metabolism--ME; *Oligonucleotides, Antisense --Pharmacology--PD; *Oxygen--Toxicity--TO; Cell Line; Cell Survival--Drug Effects--DE; Cytomegalovirus; Drug Resistance; Enzyme Inhibitors --Pharmacology--PD; Fibroblasts; Genetic Vectors; Hamsters; Heme Oxygenase (Decyclizing)--Biosynthesis--BI; Kinetics; Lipid Peroxidation--Drug...

Chemical Name: heme oxygenase-1; (Heme Oxygenase (Decyclizing); (Antioxidants; (Enzyme Inhibitors; (Genetic Vectors; (Metalloporphyrins; (Oligonucleotides, Antisense; (Recombinant Proteins; (tin mesoporphyrin; (Oxygen

9/3,K,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09226538 96180288

Bcl-2 inhibits retinoic acid-induced apoptosis during the neural differentiation of embryonal stem cells.

Okazawa H; Shimizu J; Kamei M; Imafuku I; Hamada H; Kanazawa I
Department of Neurology, Faculty of Medicine, University of Tokyo, Japan.

J Cell Biol (UNITED STATES) Mar 1996, 132 (5) p955-68, ISSN 0021-9525 Journal Code: HMV
Languages: ENGLISH
Document type: JOURNAL ARTICLE

We report here that all trans-retinoic acid (RA), a classical morphogen, induces apoptosis during the neural differentiation of the embryonic stem cell line P19. The apoptotic cells showed, in addition to DNA cleavage, typical morphological changes including chromatin condensation, nuclear fragmentation, and cytoplasmic vacuolation. These apoptotic changes became obvious by 12 h after the addition of RA. The endogenous expression of bcl-2 in surviving cells was down-regulated during this process, and the compelled expression of bcl-2 by retroviral vectors reduced the number of apoptotic cells. Apoptosis was partially inhibited by adding antisense oligonucleotides against RA receptors (RARs) simultaneously or by transfecting a plasmid vector flanked with a RA-responsive element. Antisense oligonucleotides against retinoid X receptors (RXRs), the receptors for 9 cis-RA, did not inhibit apoptosis induced by all trans-RA. Cycloheximide and actinomycin D, inhibitors of protein and RNA syntheses, respectively, suppressed apoptosis. No changes were seen in the expression of tumor necrosis factors, their receptors, Fas, FasL, p53, or c-myc, molecules which have been suggested to participate in the apoptotic process. Addition of neurotrophins to the culture medium did not affect apoptosis. These findings suggest that the signals themselves, promote expression of molecules essential for apoptosis. Furthermore, we observed that RA induced apoptosis of cerebral neurons from murine embryos in primary culture, which suggests that RA might participate in cell death which occurs during neural development.

Bcl-2 inhibits retinoic acid-induced apoptosis during the neural

differentiation of embryonal stem cells.
Mar 1996,
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...the culture medium did not affect apoptosis. These findings suggest that the signals themselves, promote expression of molecules essential for apoptosis. Furthermore, we observed that RA induced apoptosis of cerebral neurons...
; Base Sequence; Blotting, Northern; Brain--Cytology--CY; Cell Differentiation; Cell Line; Gene Expression Regulation, Developmental ; Genetic Vectors; Mice; Molecular Sequence Data; Nervous System--Cytology --CY; Nervous System--Drug Effects--DE; Neurons--Drug Effects--DE; Neurons--Pathology--PA; Oligonucleotides, Antisense; Polymeras e Chain Reaction; Signal Transduction; Stem Cells--Drug Effects--DE
Chemical Name: Genetic Vectors; (Oligonucleotides, Antisense; (Proto-Oncogene Proteins c-bcl-2; (Proto-Oncogene Proteins; (Tretinoin

9/3,K,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09210210 97462792
Central overexpression of the TRH precursor gene induces hypertension in rats: antisense reversal.
Garcia SI; Porto PI; Alvarez AL; Martinez VN; Shaurli D; Finkielman S; Pirola CJ
Departamento de Sustancias Vasoactivas, Instituto de Investigaciones Medicas A. Lanari, Facultad de Medicina, Universidad de Buenos Aires, Argentina.
Hypertension (UNITED STATES) Sep 1997, 30 (3 Pt 2) p759-66,
ISSN 0194-911X Journal Code: GK7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Extrahypothalamic TRH participates in cardiovascular regulation and spontaneous hypertension of the rat. To investigate whether an increase in central TRH activity produces hypertension we studied the effect of the preTRH overproduction induced by I.C.V. transfection with a naked eukaryotic expression plasmid vector which encodes preTRH (pCMV-TRH). Northern blot analysis and RT-PCR showed that pCMV-TRH was transcribed in vitro and in vivo. At 24, 48, and 72 hours, pCMV-TRH (100 microg) in a significant and dose-dependent manner increased 37%, 84%, and 49%, respectively, the diencephalic TRH content and SABP (42+/-3, 50+/-2, and 22+/-2 mm Hg, respectively) with respect to the vector without the preTRH cDNA insert (V[TRH(-)]) as measured by RIA and the plethysmographic method, respectively, in awake animals. In addition, using immunohistochemistry we found that the increase of TRH was produced in circumventricular areas where the tripeptide is normally located. To further analyze the specificity of these effects we studied the actions of 23-mer sense (S), antisense (AS), and 3'self-stabilized sense (Ss) and antisense (ASs) phosphorothioate oligonucleotides against the initiation codon region. Only ASs inhibited the increase of TRH content and SABP induced by pCMV-TRH treatment. In addition,

pCMV-TRH-induced hypertension seems not to be mediated by central Ang II or serum TSH. To summarize, central TRH overproduction in periventricular areas induced by I.C.V. transfection produces hypertension in rats which is reversed by specific antisense treatment. This model may help in testing effective antisense oligodeoxynucleotides against other candidate genes.

Sep 1997,

... effect of the preTRH overproduction induced by I.C.V. transfection with a naked eukaryotic expression plasmid vector which encodes preTRH (pCMV-TRH). Northern blot analysis and RT-PCR showed that pCMV-TRH ...

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Descriptors: Brain--Metabolism--ME; *Gene Expression Regulation; *Hypertension--Etiology--ET; *Oligonucleotides, Antisense --Pharmacology--PD; *Protein Precursors--Genetics--GE; *Protirelin --Genetics--GE

Chemical Name: Oligonucleotides, Antisense; (Protein Precursors; (Protirelin

9/3,K,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09166388 97407625

ETS-1 induces increased expression of erythroid markers in the pluripotent erythroleukemic cell lines K562 and HEL.

Clausen PA; Athanasiou M; Chen Z; Dunn KJ; Zhang Q; Lautenberger JA; Mavrothalassitis G; Blair DG

Division of Basic Sciences, National Cancer Institute, Frederick, MD 21702-1201, USA.

Leukemia (ENGLAND) Aug 1997, 11 (8) p1224-33, ISSN 0887-6924 Journal Code: LEU

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Members of the ETS gene family are known to be expressed in hematopoietic tissues and cell lines, and there is increasing evidence that ETS proteins may play a role in normal hematopoietic cell development. We demonstrate that ETS-1 can contribute to the development of an erythroid phenotype in vitro. The pluripotent erythroleukemic K562 and HEL cell lines express messages for a number of ETS genes, but only c-ETS-1 levels are elevated in response to treatment with hemin or cytosine arabinofuranoside (Ara-C), agents which induce erythroid differentiation. Furthermore, ETS-1 antisense oligonucleotides inhibit hemoglobinization of cells treated with Ara-C or hemin, and K562 and HEL cells infected with retrovirus expressing the c-ETS-1 gene exhibit a significant increase in erythroid character (as indicated by benzidine staining for hemoglobin (Hb) and surface marker analysis), a dramatic increase in responsiveness to hemin or Ara-C, and a decreased rate of proliferation (20-40% of control rates). In contrast, infection with virus expressing ETS-2 or vector sequences only causes no detectable changes in the proliferation or erythroid character of either the HEL or K562 cell lines. These data indicate a role for ETS-1 in erythroid differentiation.

ETS-1 induces increased expression of erythroid markers in the pluripotent erythroleukemic cell lines K562 and HEL.

Aug 1997,

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... a decreased rate of proliferation (20-40% of control rates). In contrast, infection with virus expressing ETS-2 or vector sequences only causes no detectable changes in the proliferation or erythroid character of either the...
...; Effects--DE; Cell Division--Drug Effects--DE; Cytarabine --Pharmacology--PD; Erythropoiesis--Drug Effects--DE; Gene Expression Regulation, Developmental--Drug Effects--DE; Hemin--Pharmacology--PD; Hemoglobins--Biosynthesis--BI; Oligonucleotides, Antisense --Pharmacology--PD; RNA, Messenger--Genetics--GE; Tumor Cells, Cultured
Chemical Name: proto-oncogene protein ets; (proto-oncogene protein ets-2; (Hemoglobins; (Oligonucleotides, Antisense; (Proto-Oncogene Proteins; (RNA, Messenger; (Trans-Activators; (Transcription Factors; (Cytarabine; (Hemin

9/3,K,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09156858 97386387
Downregulation of cyclin G1 expression by retrovirus-mediated antisense gene transfer inhibits vascular smooth muscle cell proliferation and neointima formation.
Zhu NL; Wu L; Liu PX; Gordon EM; Anderson WF; Starnes VA; Hall FL
USC Gene Therapy Laboratories, Childrens Hospital of Los Angeles, and the University of Southern California School of Medicine, 90027, USA.
Circulation (UNITED STATES) Jul 15 1997, 96 (2) p628-35, ISSN 0009-7322 Journal Code: DAW
Contract/Grant No.: GM-49715, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
BACKGROUND: The contemporary treatment of coronary athero-occlusive disease by percutaneous transluminal coronary angioplasty is hampered by maladaptive wound healing, resulting in significant failure rates. Morbid sequelae include smooth muscle cell (SMC) hyperplasia and restenosis due to vascular neointima formation. METHODS AND RESULTS: In this study, we examined the inhibitory effects of a concentrated retroviral vector bearing an antisense cyclin G1 gene on aortic SMC proliferation in vitro and on neointima formation in vivo in a rat carotid injury model of restenosis. Retroviral vectors bearing an antisense cyclin G1 construct inhibited the proliferation of transduced aortic SMCs in 2- to 6-day cultures, concomitant with down-regulation of cyclin G1 protein expression and decreased [3H]thymidine incorporation into DNA. Morphological examination showed evidence of cytolysis, giant syncytia formation, and apoptotic changes evidenced by overt cell shrinkage, nuclear fragmentation, and specific immunostaining of nascent 3'-OH DNA ends generated by endonuclease-mediated DNA fragmentation. Pronounced "bystander effects" including neighboring cells were noted in aortic SMCs transduced with the antisense cyclin G1 vector, as determined by quantitative assays and fluorescent labeling of nontransduced cells. In an in vitro

tissue injury model, the proliferation and migration of antisense cyclin G1 vector-transduced aortic SMCs were inhibited. Moreover, in vivo delivery of high-titer antisense cyclin G1 vector supernatant to the balloon-injured rat carotid artery in vivo resulted in a significant reduction in neointima formation. CONCLUSIONS: These findings represent the first demonstration of the inhibitory effects of an antisense cyclin G1 retroviral vector on nonneoplastic cell proliferation. Taken together, these data affirm the potential utility of antisense cyclin G1 constructs in the development of novel gene therapy approaches to vascular restenosis.

Downregulation of cyclin G1 expression by retrovirus-mediated antisense gene transfer inhibits vascular smooth muscle cell proliferation and neointima formation.
Jul 15 1997,
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...Descriptors: Carotid Stenosis--Pathology--PA; *Cell Movement--Genetics --GE; *Cyclins--Genetics--GE; *Gene Therapy; *Gene Transfer; * Oligonucleotides, Antisense--Genetics--GE...; TH; Cell Division --Genetics--GE; Cells, Cultured; Cyclins--Biosynthesis--BI; Down-Regulation (Physiology); Genetic Vectors; Oligonucleotides, Antisense --Administration and Dosage--AD; Rats; Rats, Wistar; Retroviridae; Tunica Intima--Metabolism--ME; Tunica Intima--Pathology...
Chemical Name: cyclin G1; (Cyclins; (Genetic Vectors; (Oligonucleotides, Antisense

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? s peripheral(w)type(5n)benzodiazepine(5n)receptor

          599165  PERIPHERAL
         1848185  TYPE
           44578  BENZODIAZEPINE
         1124156  RECEPTOR
      S1     536  PERIPHERAL(W)TYPE(5N)BENZODIAZEPINE(5N)RECEPTOR
? s antisense

      S2   38974  ANTISENSE
? s s1 and s2

            536  S1
          38974  S2
      S3      9  S1 AND S2
? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S4      4  RD (unique items)
? t s4/3,k,ab/1-4


 4/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09450641   98157936
      Effects of peripheral-type benzodiazepine
receptor antisense knockout on MA-10 Leydig cell proliferation
and steroidogenesis.
  Kelly-Hershkovitz E; Weizman R; Spanier I; Leschiner S; Lahav M;
Weisinger G; Gavish M
  Department of Pharmacology, The Bruce Rappaport Faculty of Medicine,
Technion-Israel Institute of Technology, 31096 Haifa, Israel.
  J Biol Chem (UNITED STATES) Mar 6 1998, 273 (10) p5478-83, ISSN
0021-9258 Journal Code: HIV
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  The peripheral-type benzodiazepine receptor (PBR)
is not only widely expressed throughout the body, but it is also
genetically conserved from bacteria to humans. Many functions have been
attributed to it, but its primary role remains a puzzle. In the current
study, we stably transfected cultures of MA-10 Leydig cells with either
control or 18-kDa PBR antisense knockout plasmids. The
antisense knockout vector was driven by the human enkephalin
promoter, which contains two cAMP response elements, such that cAMP
treatment of transfected cells could superinduce 18-kDa PBR antisense
RNA transcription and, hence, down-regulate endogenous 18-kDa PBR mRNA
levels. Control and knockout MA-10 cell lines were then compared at the
level of receptor binding, thymidine incorporation, and steroid
biosynthesis. Eighteen-kilodalton PBR knockout reduced the maximal binding
capacity of tritium-labeled PBR ligands, and the affinity of receptors to
the ligands remained unaltered. Additionally, 24-h accumulation of
progesterone was lower in the knockout cells. Exposure of the two cell
types to 8-bromo-cAMP resulted in a robust increase in steroid production.
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However, a complex pattern of steroid accumulation was observed, in which further progestin metabolism was indicated. The later decline in accumulated progesterone as well as the synthesis of androstenedione were different in the two cell types. At the level of cell proliferation, reduction of 18-kDa PBR mRNA showed no effect. Thus, we conclude that the 18-kDa PBR may have a more important role in steroidogenesis than in proliferation in this Leydig cell line.

Effects of **peripheral-type benzodiazepine receptor antisense** knockout on MA-10 Leydig cell proliferation and steroidogenesis.

The **peripheral-type benzodiazepine receptor** (PBR) is not only widely expressed throughout the body, but it is also genetically conserved...

...stably transfected cultures of MA-10 Leydig cells with either control or 18-kDa PBR **antisense** knockout plasmids. The **antisense** knockout vector was driven by the human enkephalin promoter, which contains two cAMP response elements, such that cAMP treatment of transfected cells could superinduce 18-kDa PBR **antisense** RNA transcription and, hence, down-regulate endogenous 18-kDa PBR mRNA levels. Control and knockout...

Descriptors: DNA, **Antisense**--Pharmacology--PD; *Leydig Cells --Metabolism--ME; *Receptors, GABA-A--Antagonists and Inhibitors--AI

Chemical Name: Benzodiazepinones; (DNA, **Antisense**; (Isoquinolines; (Plasmids; (Receptors, GABA-A; (RNA, Messenger; (Ro 5-4864; (Clonazepam; (Progesterone; (Algestone; (Cyclic AMP...

4/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08462688 96070187

Involvement of peripheral-type benzodiazepine receptors in the intracellular transport of heme and porphyrins.

Taketani S; Kohno H; Furukawa T; Tokunaga R
Department of Hygiene, Kansai Medical University, Osaka.

J Biochem (Tokyo) (JAPAN) Apr 1995, 117 (4) p875-80, ISSN 0021-924X Journal Code: HIF
Languages: ENGLISH
Document type: JOURNAL ARTICLE

To investigate the involvement of peripheral-type benzodiazepine receptors (PBR) in heme metabolism, we examined the interaction of [55Fe]heme with PBR. Transfection of the cloned mouse PBR-isoquinoline carboxamide-binding protein (PBR/IBP) cDNA into monkey kidney Cos-1 cells resulted in a 2.5-fold increase in [55Fe]hemin binding sites, concomitant with the increase in [3H]PK11195 binding sites, as compared with those seen in **antisense** PBR/IBP cDNA-transfected cells. The binding of hemin to the transfected receptors exhibited a relatively high affinity with a Kd of 12 nM, and was inhibited by several benzodiazepine ligands, including PK11195, Ro 5-4864, diazepam and protoporphyrin IX. When mouse liver mitochondria were incubated with [55Fe]hemin, the binding to PBR had a Kd of 15 +/- 1.8 nM. The Bmax of [55Fe]hemin binding to the mitochondria was 6.88 +/- 0.76 pmol/mg of protein, a value consistent with that of [3H]PK11195 binding, with a lower affinity. Coproporphyrinogen III, a precursor porphyrin produced in the cytosol, is translocated into mitochondria, then is converted to protoporphyrinogen IX; this conversion decreased in the presence of benzodiazepine ligands. To examine whether this decrease was related to a decrease in the binding of coproporphyrinogen to the mitochondria, the effects of benzodiazepines on the binding of coproporphyrinogen were examined. As the binding was dose-dependently inhibited by PK11195, Ro 5-4864, and diazepam, porphyrins are likely to be endogenous ligands for PBR. We propose that PBR play a role in the intracellular transport of porphyrins and heme.

... concomitant with the increase in [3H]PK11195 binding sites, as

compared with those seen in **antisense** PBR/IBP cDNA-transfected cells. The binding of hemin to the transfected receptors exhibited a...
Chemical Name: Carrier Proteins; (Iron Radioisotopes; (Porphyrins; (Protoporphyrins; (Receptors, GABA-A; (bovine **peripheral-type benzodiazepine receptor** isoquinoline-binding protein; (Heme; (Hemin; (protoporphyrinogen; (Iron

4/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07984522 94350958
The polypeptide diazepam-binding inhibitor and a higher affinity mitochondrial **peripheral-type benzodiazepine receptor%**
%% sustain constitutive steroidogenesis in the R2C Leydig tumor cell line.
Garnier M; Boujrad N; Ogwuegbu SO; Hudson JR Jr; Papadopoulos V
Department of Cell Biology, Georgetown University Medical Center, Washington, D.C. 20007.
J Biol Chem (UNITED STATES) Sep 2 1994, 269 (35) p22105-12, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: DK-43358, DK, NIDDK; HD-01031, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The polypeptide diazepam binding inhibitor (DBI) and drug ligands for the mitochondrial peripheral-type benzodiazepine receptor%
%% (PBR) have been shown to regulate cholesterol transport, the rate-determining step in steroidogenesis, in hormone-responsive steroidogenic cells including the MA-10 Leydig tumor cells. The present study was designed to characterize the role of DBI and PBR in the R2C rat Leydig tumor constitutive steroid-producing cell model. Both DBI and PBR were present in R2C cells. R2C cell treatment with a cholesterol-linked phosphorothioate oligodeoxynucleotide **antisense** to DBI, but not sense, resulted in the reduction of DBI levels and a concomitant dramatic decrease of the amount of progesterone produced. These observations strongly suggested that DBI was important in maintaining constitutive steroidogenesis in R2C cells. Radioligand binding assays revealed the presence of a single class of PBR binding sites with an affinity 10 times higher (Kd approximately 0.5 nM) than that displayed by the MA-10 PBR (Kd approximately 5 nM). Photolabeling of R2C and MA-10 cell mitochondria with the photoactivatable PBR ligand [3H]1-(2-fluoro-5-nitrophenyl)-N-methyl-N-(1-methyl-propyl)-3- isoquinolinecarboxamide showed that the M(r) 18,000 PBR protein was specifically labeled. This indicates that the R2C cells express a PBR protein which has properties similar to the MA-10 PBR. Chemical crosslinking studies of purified metabolically radiolabeled DBI to mitochondria provided direct evidence that DBI specifically binds to the M(r) 18,000 PBR protein. Moreover, DBI and a PBR synthetic ligand were able to increase steroid production in isolated R2C cell mitochondria which express the 5 nM affinity receptor. However, mitochondrial PBR binding was increased by 6-fold upon addition of the post-mitochondrial fraction, suggesting that a cytosolic factor modulates the binding properties of PBR in R2C cells and is responsible for the 0.5 nM affinity receptor seen in intact cells. In conclusion, these data demonstrate that DBI plays a key role in maintaining R2C constitutive steroidogenesis by binding to the mitochondrial higher affinity PBR which promotes a continuous supply of cholesterol to the inner mitochondrial side chain cleavage cytochrome P450.

The polypeptide diazepam-binding inhibitor and a higher affinity mitochondrial **peripheral-type benzodiazepine receptor%**
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... PBR were present in R2C cells. R2C cell treatment with a cholesterol-linked phosphorothioate oligodeoxynucleotide **antisense** to DBI, but not sense, resulted in the reduction of DBI levels and a concomitant...

4/3,K,AB/4 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

11800023 BIOSIS NO.: 199900046132
Effects of **peripheral-type benzodiazepine receptor** **antisense** knockout on MA-10 Leydig cell proliferation and steroidogenesis.
AUTHOR: Gavish M(a); Weizman R; Spanier I(a); Leschiner S(a); Lehav M(a); Weisinger G(a); Kelly-Hershkovitz E(a)
AUTHOR ADDRESS: (a)Bruce Rappaport Fac. Med., Technion-Israel Inst. Technol., 31096 Haifa**Israel
JOURNAL: Society for Neuroscience Abstracts 24 (1-2):p347 1998
CONFERENCE/MEETING: 28th Annual Meeting of the Society for Neuroscience, Part 1 Los Angeles, California, USA November 7-12, 1998
SPONSOR: Society for Neuroscience
ISSN: 0190-5295
RECORD TYPE: Citation
LANGUAGE: English

Effects of **peripheral-type benzodiazepine receptor** **antisense** knockout on MA-10 Leydig cell proliferation and steroidogenesis.
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...**peripheral-type benzodiazepine receptor**--...

...**antisense** knockout plasmids